Antioxidant activity of mango wine in \textit{in vitro} and \textit{in vivo}
7.1 INTRODUCTION

The biological process of wine-making is the result of a series of biochemical transformations brought about by the action of several enzymes from different microorganisms, especially yeasts, which are responsible for the principal part of the process, alcoholic fermentation. The most important transformation that takes place in the grape juice during vinification is the alcoholic fermentation of the sugars, especially hexoses (glucose and fructose), that mainly result in ethanol and carbon dioxide production and the generation of a large number of by-products.

Red wine has a special role in human nutrition because it is an important source of antioxidants. Essential wine components responsible of its healthy properties are polyphenols, those compounds which also responsible for its important sensory characteristics such as color, astringency and bitterness. Trans-resveratrol is one of the most investigated among grape polyphenols, since its discovery in red wine and its beneficial effects include anticancer (Damianaki et al., 2000), antioxidant (Frankel et al., 1995), and anti-amyloidogenic (Marambaud et al., 2005) activities; moreover, its capacity to extend the lifespan of diverse species, including Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila melanogaster, has been recently demonstrated (Baur et al., 2006). Although a great number of studies have implicated its significant role in disease prevention, the mode of action of trans-resveratrol in vivo is unclear. Therefore several researchers suggest that the benefits associated with red wine consumption are due to whole antioxidant compounds of this beverage. In fact, also anthocyanins, tannins, quercetin, and the flavan-3-ols, (+)-catechin, and (-)-epicatechin exhibit a biological activity (Frankel et al., 1995). Because of the well-documented large influence of polyphenols on wine sensory (Lesschaeve and Noble 2005) and nutritive quality, numerous studies were focused on the techniques able to influence the release of these compounds from grape during vinification.

7.1.1 Moderate alcohol consumption and plasma lipids: Epidemiological studies have demonstrated that regular consumption of moderate amounts of wine reduces the incidence of death from atherosclerosis and coronary artery disease (Kannel and
Ellioson, 1996). This effect may be partly related to the ability of ethanol to increase the plasma concentration of the cardio-protective high-density lipoprotein (HDL) (Gaziano et al., 1993). In addition, chronic exposure to ethanol induces upregulation of antioxidant enzymes of rat liver and kidney (Roig et al., 1999), but beneficial effects of red wine surpass those merely attributable to alcohol (Gronbaeck et al., 1995). This enhanced protection may be due to the presence of polyphenols, strong antioxidant compounds particularly abundant in red wine (McDonald et al., 1998). These compounds are synthesized in the epidermal cells and in the seed of the grape, what explains why the white wine that is not made in the presence of these components shows low levels of polyphenols (Jamroz and Beltowski, 2001) in contrast with red wine that uses the skin of the grape in the process of fermentation. Polyphenols exert a large array of biological actions, such as free radical-scavenging, metal chelation and enzyme modulation (Pietta et al., 1998), as well as reduction of the susceptibility of low density lipoproteins (LDL) to oxidation both in vitro (Bertelli et al., 1996) and in vivo (Nigdikar et al., 1998). Also, polyphenols may participate in the regulation of vascular tone (Adriantsitohaina, 1999) or in the inhibition of platelet aggregation (Keevil et al., 2000). Clinical studies have shown a significant rise in serum antioxidant capacity after consumption of red wine (Durak et al., 1999), an effect also reported in rats (Rodrigo et al., 2002) which could reinforce the systemic endogenous antioxidant defense system.

Wines contain a number of polyphenolic antioxidants including phenolic acids (p-coumaric, cinnamic, caffeic, gentistic, ferulic, and vanillic acids), proanthocyanidins, catechin, and epicatechin quercetin. Red wines are also rich in resveratrol (3,5,4-trihydroxystilbene), which is generally present in the amount of 1-10 mg/L depending on the variety of red wine. In most U.S. varieties of red wine, the concentration of resveratrol rarely exceeds 1 mg/L (Jeandet et al., 1995). Italian red wines contain much higher amounts of resveratrol, ranging from 5 to 10 mg/L (Hattori et al., 2002). Among the components of red wine, resveratrol has received much attention as supplying most of the cardioprotective property of red wine. For example, although resveratrol is a poor antioxidant in vitro, it functions as a potent antioxidant in vivo (Hall, 2003). Similar to red wine, resveratrol can increase HDL, lower LDL, exert anti-inflammatory action, and
function as a potent vasodilator through its ability to stimulate nitric oxide (NO) synthesis (Falchi et al., 2006).

Evidence is rapidly emerging supporting the cardioprotective role of white wine. Initial studies focused on cardioprotection with red wines only because white wines contain very little to no resveratrol. The reason for this is that red wines are produced from the grapes that include their skins, which contain resveratrol, whereas white wines are made from grapes without their skins. Nevertheless, one of our earlier studies performed by feeding white wine to rats demonstrated cardioprotective abilities of certain varieties of white wines (Cui et al., 2002a). Consequently, several other studies also found cardioprotective abilities of white wines (Cui et al., 2002b).

Although white wines do not contain resveratrol to any appreciable amount, they contain other cardioprotective antioxidants such as cinnamic acid, tyrosol, and hydroxytyrosol. Tyrosol and hydroxytyrosol are phenolic compounds that are also found in olive oil, which are present in olives either as free or conjugated forms as steroids or aglycones (Covas et al., 2003). Tyrosol and hydroxytyrosol possess several cardioprotective properties including their abilities to protect LDL against oxidation (Petroni et al., 1995), inhibit platelet aggregation (Carluccio et al., 2003), prevent formation of pro-inflammatory agents by activated leukocytes (Bertelli et al., 2002), and inhibit endothelial expression of tissue factor (Javadov et al., 2008). Although alcohol could also provide cardioprotection by reducing LDL and increasing HDL as well as by exerting preconditioning-like effect, it appears to be unlikely that the small amount of alcohol present in our samples would exert any significant cardioprotection as our results indicated no improvement in cardioprotection with control alcohol over the baseline control. Since many decades, it has been an established fact that diet containing cholesterol creates atherosclerotic changes in the arteries of laboratory animals and humans. Currently, researchers throughout the world agree that cholesterol is also the “building material” for atherosclerotic plaques in humans and elevated levels of total cholesterol, low-density cholesterol (LDL-C), triglycerides, apolipoproteins B and C-III and reduced levels of high-density cholesterol (HDL-C)
and apolipoprotein A-I are major risk factors for this disease (Castelli et al., 1986; Kwiterovich et al., 1992).

Fig. 7.1. Mechanisms by which moderate alcohol consumption exerts its beneficial effects on the cardiovascular system (CHD = coronary heart disease; HDL = high density lipoprotein) (Source: Hines and Rimm, 2001).

Moderate consumption of alcoholic beverages leads first to an increase in HDL-cholesterol and has little effect on total and LDL-cholesterol (Hashimoto et al., 2001; Renaud and de Lorgeril, 1992). However, in experiments on laboratory animals it was demonstrated that diets supplemented with moderate quantities of alcoholic beverages led to decreased levels of plasma total cholesterol, LDL-C, triglycerides, total phospholipids and total cholesterol in liver and an increased level of HDL-C in animals fed added cholesterol (Gorinstein et al., 1998a, 1998b, 2001). The same tendency was observed in clinical investigations of patients suffering from hypercholesterolemia that moderate consumption of alcoholic beverages led to a decrease in the level of total cholesterol, LDL-C, triglycerides and an increase of HDL-cholesterol (Gorinstein et al.,
1997, 2003). Contrary to the above, increase of the levels of HDL-C after moderate alcoholic consumption is not disputable. An increase in the level of HDL-C was found in experiments on laboratory animals and also in investigations of patients with and without hypercholesterolaemia. (Gorinstein et al., 2000, 2001; Sillanaukee et al., 2000; Hashimoto et al., 2001). Sillanaukee et al., (2000) have reported that HDL-C (the HDL3 subfraction) was higher in those drinking daily 20-40 g of alcohol. It was also found that the frequency of drinking was associated with a higher level of HDL-C (Hashimoto et al., 2001). It was shown by Rozenberg et al. (2003) that paraoxonase (PON), an organophosphate enzyme (HDL associated enzyme) is related to suppression of atherosclerosis. Fuhrman and Aviram (2002) have shown that paraoxonase is associated physically with HDL, and its activity has been related inversely to the risk of cardiovascular diseases and also, PON can hydrolyze specific lipid peroxides in oxidized lipoproteins and in atherosclerotic lesions. Fuhrman and Aviram (2002) have demonstrated that consumption of wine flavonoids preserves PON activity by reducing the oxidative stress in apolipoprotein E-deficient mice, thereby contributing to paraoxonase hydrolytic activity on lipid peroxides in oxidized lipoproteins and atherosclerotic lesions. Paraoxonase inhibits macrophage cholesterol biosynthesis and atherogenesis probably through its phospholipase-A(2)-like activity and it was shown also in investigations of humans that paraoxonase activity is related inversely to the risk of cardiovascular diseases. Van der Gaag et al. (1999) found that the paraoxonase activity was higher after intake of wine, beer and spirits than after water consumption and this investigation suggest that increased serum paraoxonase may be one of the biological mechanisms underlying the reduced coronary heart disease risk in moderate alcohol consumers. It was also showed that PON, have antioxidant properties, inhibiting LDL oxidation (Aviram et al., 2005) and Sierksma et al. (2002a) reported the kinetics of alcohol-induced (30-40 g/day of beer) increases in apo A-I, HDL-C and paraoxonase activity and found that the serum apo A-I, HDL cholesterol and paraoxonase activity were increased significantly during three weeks of moderate alcohol consumption compared with no alcohol consumption and concluded that the increased serum HDL-C and PON activity may be a mechanism of action not only in healthy middle-aged men.
but also in postmenopausal women, underlying the reduced coronary heart disease risk in moderate drinkers.

7.1.2 Moderate alcohol consumption and plasma antioxidant activity: It was evidently found that atherosclerosis is an oxidative disease (Steinberg et al., 1989; Witztum and Steinberg, 1991). Further, Perez et al. (2002) have proved that oxidative stress is a central mechanism for the pathogenesis of atheromaemic heart disease and atherogenesis. Some evidence suggests that one of the important mechanisms predisposing to development of atherosclerosis is the oxidation of cholesterol–rich LDL-C particles (Witztum and Steinberg, 1991). Oxidation of LDL-C enhances its atherogenicity and facilitates penetration of lipids into the arterial wall and according Steinberg et al., 1989; Witztum and Steinberg, (1991), prevention of atherosclerosis is a fight against LDL-C oxidation. In most western industrial countries intake of food products containing high quantities of cholesterol is correlated positively with morbidity and mortality from coronary artery disease (CAD) (Renaud and de Lorgeril. 1992). However, total consumption of saturated fats in France is equal to that of other developed countries, while French mortality from CAD is only one-third of the average and this phenomenon is known as the French paradox for CAD (Renaud and de Lorgeril. 1992; Perez et al. (2002)). The distinguishing feature of the French diet is regular consumption of red wine with meals. Therefore, it was supposed that regular consumption of red wine could explain why French mortality from CAD is only one-third of the average in other developed countries (Renaud and de Lorgeril. 1992; Perez et al. (2002)). The antioxidant properties of red wine were confirmed in vitro by Frankel et al. 1993a, 1993b) have showed that oxidation of human low-density lipoprotein is inhibited by the phenolic substances of red wine.

Further experiments on laboratory animals and in investigation of humans, it showed that red wine, white wine and beer increase plasma antioxidant activity (Gorinstein et al., 1997, 1998a, 1998b, 2001). However, in these in vitro studies the degree of antioxidant activity was different, among the three widely used alcoholic beverages (red wine, white wine and beer) the highest content of total polyphenols is in red wine and the lowest in beer. Also Paganga et al. (1999) have proved that the
antioxidant activities of one glass of red wine (150 mL) is equivalent to 3.5 glasses of beer (500 ml) and equivalent to 12 glasses of white wine. Therefore, the concentration of total polyphenols not only plays a role, but also the content of some essential phenolics. Among the three widely used alcoholic beverages (red wine, white wine and beer), red wine has the highest content of total polyphenols and the highest content of some essential phenolics. Therefore, most of the researchers claim that red wine may be the best choice for consumers (Klatsky and Armstrong, 1993).

7.1.3 Moderate alcohol consumption and plasma anticoagulant activity: Currently evidences from epidemiological, experimental and clinical studies suggest a protective effect against the development of atherosclerosis with moderate consumption of alcoholic beverages. The exact nature of the protective effect remains to be established. However, mechanisms including alterations in haemostatic variables is being recognized increasingly as contributory (Wollin and Jones, 2002). Thrombosis of the coronary arteries is the main reason for fatal cases from myocardial infarctions due to coronary atherosclerosis (Brosins and Roberts, 1983). As discussed in the above section, moderate consumption of alcoholic beverages lowers the risk and the death rate from cardiovascular diseases. Therefore, the decrease in the death rate could not be achieved without a beneficial influence on haemostatic risk factors (Dimmitt et al., 1998). Many investigations have demonstrated that consumption of alcoholic beverages is correlated with haemostatic risk factors (Siersksma et al., 2001; Mukamal et al., 2001 Buemann et al., 2002). It was found that platelet aggregation is inhibited by moderate alcohol consumption (Mezzano et al., 2001). According to Renaud and de Lorgeril, (1992) this is the main reason for the low death rate from coronary atherosclerosis in France. It was also found in a clinical investigation that moderate beer consumption leads to a significant decrease in the prothrombin time (Gorinstein et al., 1997b). Fibrinogen is one of the plasma circulating proteins. This protein is synthesized in liver and circulates in plasma at a concentration of 200-400 mg/dl. Fibrinogen plays an important role in blood clotting, fibrinolysis, cellular and matrix interactions. Evidence links fibrinogen with coronary atherosclerosis and blood coagulation (Mosesson et al., 2001). It was reported that fibrinogen levels predicted cardiovascular events
independently of traditional risk factors and it was demonstrated that moderate drinking leads to a decrease in the plasma circulating fibrinogen concentration (Mennen et al., 1999; Wang et al., 1999). Wang et al. (1999) have shown that daily consumption of moderate amounts of ethanol decreases circulating levels of fibrinogen by 18–20%. Also, some structural changes in plasma circulating fibrinogen after short-term moderate beer consumption were found using electrophoresis and spectroscopy. In order to evaluate the status of the plasma anticoagulant activity Factor VIIag, Factor VIIc and PAI were studied. A decrease was found in all these tests after moderate beer consumption (Gorinstein et al., 2000, 2001, 2003). Mukamal et al. (2001) have measured the levels of fibrinogen, plasma viscosity, von Willebrand factor, factor VII, plasminogen activator inhibitor antigen-I and tissue plasminogen activator antigen in a cross-sectional analysis of 3223 enrolled in the Framingham offspring study. They have found that light-to-moderate alcohol consumption is associated with lower levels of coagulatory activity, but higher intake is associated with impaired fibrinolytic potential. Hence, epidemiological and clinical investigations have shown that only moderate consumption of alcoholic beverages influences plasma anticoagulant activity favourably.

7.1.4 Moderate alcohol consumption and other biochemical changes: The positive influence of moderate alcohol consumption on plasma lipids, plasma antioxidant and plasma anticoagulant activity is well known. However, moderate consumption of alcoholic beverages leads to some other biochemical changes. It was found in a randomized, diet-controlled interventional study that moderate alcohol consumption reduces plasma C-reactive protein levels (Sierksma et al., 2002b). In another investigation it was demonstrated that red wine consumption influences positively pre-incubation of vascular smooth muscle cells, inhibits ligand binding and the subsequent tyrosine phosphorylation of the platelet-derived growth factor beta receptor, which plays a critical role in the pathogenesis of atherosclerosis (Rosenkranz et al., 2002). There is evidence that consumption of alcoholic beverages influences positively synthase of nitric oxide, which plays a critical role in cardiovascular protection and is the responsible cardioprotective protein. Leikert et al. (2002) have shown that red wine
polyphenols increase endothelial nitric oxide synthase and subsequent endothelial nitric oxide release. Increased endothelial nitric oxide activity may antagonize the development of endothelial dysfunction and atherosclerosis, a hypothesis that supports the view that red wine may indeed have long-term protective cardiovascular properties mediated by its polyphenols.

7.1.5 Ethanol induced oxidative stress: Oxidative stress is defined as an imbalance (the failure of the cell's defense against the deleterious effects of harmful agents) between higher cellular levels and reactive oxygen species (ROS) e.g. superoxide and hydroxyl radicals and cellular antioxidant defense (Reinke, 2002). Oxidative stress is well known to cause lipid peroxidation, which induces disturbance of membrane organization leading to alteration of its fluidity and permeability and generating potentially toxic products, which may be mutagenic and carcinogenic or may exert pro-atherogenic and pro-inflammatory effects (Niki, 2009). Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism i.e., mitochondrial oxidations and phagocytosis. In order to scavenge ROS, various defense systems exist in blood. The cell has numerous protective defense mechanisms against various threatening factors that are induced by its adaptive mechanisms as well as by an increase in its synthetic and metabolic activities. Vitamin E (a-tocopherol) is the most important antioxidant in the lipid phase of cells (Naziroglu, 2007). Vitamin C (ascorbic acid), as well as being a free radical scavenger, also transforms vitamin E to its active form. Vitamin A (retinol) serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (Halliwell, 2006). For instance, if the target of the injury-causing agent is protein synthesis, which may result in a deficiency of structural or the other types of proteins (i.e. enzymes, pumps, transport proteins, and channels), the cell increases its synthetic activity if the protein synthesis apparatus is not damaged. If the target is energy metabolism, which may result in energy depletion, the cell increases the production of ATP via alternate pathways, such as anaerobic glycolysis, unless cellular glycogen content is depleted. These are compensatory responses of the cell to protect itself from damage and death. If the cell's defense system fails, reversible damage transforms into irreversible damage, ultimately
causing cell death. Most cells can tolerate a mild degree of oxidative stress because they have sufficient antioxidant defense capacity and repair systems that recognize and remove molecules damaged by oxidation. Cells generate energy by reducing molecular oxygen to water. During this process, small amounts of partially reduced reactive oxygen species (ROS) are produced as a byproduct of mitochondrial respiration. Some of these are free radicals that can damage many components of cells, including lipids, proteins, and nucleic acids (Hensley et al., 2000).

Physiological levels of ROS are beneficial for cells. ROS can regulate transcription by activating the transcription of specific genes and can act in the immune system as effector molecules against pathogens. Many components of the cell, including mitochondria, endoplasmic reticulum, peroxisomes, membranes, and cytosol, can be sources of ROS. In general, there is a balance between the production of ROS and cellular antioxidant agents. The accumulation of low-to-moderate levels of ROS is generally counterbalanced by the cell’s endogenous antioxidant defense system (Figure 7.2). Antioxidant agents act jointly to remove various ROS produced by free radical
reactions. Indeed, antioxidant activity may be a consequence of ROS production. If the amount of ROS increases, and if these products destroy the apparatus by which antioxidant agents are produced, the cellular defense system is eventually incapacitated. It appears that higher levels of ROS induce necrotic cell death whereas lower levels lead to apoptosis.

7.1.6 Ethanol induced hepatic damage: Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses rapidly into circulation and is distributed uniformly throughout the body. Ethanol is almost exclusively metabolized in the body by enzyme catalyzed oxidative processes. The acetaldehyde formed is further oxidized to acetate, which is then converted to carbon dioxide via the citric acid cycle. Ethanol or its metabolites can also cause auto-oxidation of the hepatic cells either by acting as a pro-oxidant or by reducing the anti-oxidant levels resulting in marked hepatotoxicity (Crawford and Balakenhoan, 1991). Lipid peroxidation and associated membrane damage is a key feature in alcoholic liver injury (Lieber, 1993). Ethanol indirectly induces alterations of membrane fluidity, channel and pumps, and ionic transients. Recently, several studies have examined the role of oxidative stress in development of alcohol mediated blood and tissue toxicity, possibly via the formation of ROS (Caliskan et al., 2010). Ethanol initiates the oxidation of mitochondrial and cytoplasmic membrane, lipids, damaging membranes and leading to the formation of ROS (Siler-Marsiglio et al., 2004). Some authors have been suggested that alcohol has been shown to induce lipid peroxidation and to decrease antioxidant such as glutathione and vitamin C level due to the generation of ROS in animal and human alcoholics (Caliskan et al., 2010).

The liver is the main target organ of ethanol toxicity; it has been demonstrated experimentally that chronic ethanol ingestion leads to an increase of lipid peroxidation products and a decrease of antioxidant factors such as glutathione (GSH) and derived enzymes. Likewise, oxidative stress has been related as the main factor implicated in alterations derived from its chronic consumption, from the Central nervous system (CNS) as well as from the peripheral nervous system (Diaz et al., 2002). There are several mechanisms involved in ethanol consumption-related liver damage; the former
results from the effects of alcohol dehydrogenase (ADH) mediated by the excessive
generation of NADH and acetaldehyde, which generates the formation of Free radicals (Lieber, 2004). Acetaldehyde increases the production of alkanes and causes an imbalance in potential cytosolic redox on altering the NAD'/NADH relationship, as occurs in the mitochondria; this redox alteration favors the production of lipoperoxides, which increase damage to the cell (Gutierrez-Salinas and Morales-Gonzalez, 2004). Acetaldehyde inactivates the enzymes, diminishing DNA repair, antibody production, and glutathione depletion, and increasing mitochondrial toxicity, endangering oxygen utilization, and increasing collagen synthesis (Lieber, 2004). Another probable mechanism of hepatic damage associated with chronic ethanol consumption is the increase in the synthesis of fatty acids and triglycerides and a decrease of the oxidation of the former, generating hyperlipidemias that leads to the development of fatty liver, in addition to inhibiting fatty acids utilization and the availability of precursors, which stimulate the hepatic synthesis of triglycerides (Tellez and Cote, 2006). Previous studies have suggested that Kupffer cells are involved in hepatic damage caused by ethanol consumption; this is due to that ethanol alters the functions of these cells, such as phagocytosis, bactericide activity, and the production of inflammatory cytokines such as Tumor growth factor alpha (TNF)-α, Interleukin 1 (IL-1), and IL-6, among others, which result in hepatic cell toxicity (Thurman, 1998). It has been reported that TNF-α and IL-1 inhibit protein synthesis in hepatocytes in rat, in addition to stimulating neutrophil migration and activation, as well as protease induction and free radicals release (Thurman, 1998). Cytokines and chemokines originated by the Kupffer cells employ autoparacrine as well as paracrine effects that initiate the defensive response in the liver, but that also promote the infiltration of inflammatory leukocytes and activate the oxidative attack response, accompanied by strong damage originating from degrading cytokines and proteins. Cellular infiltration of activated neutrophils produces oxygen free radicals and secretes other toxic mediators; additionally, these can increase the inflammatory response, causing damage and cell death (Thurman, 1998). Ethanol consumption induces changes in the mitochondrial membrane, such as Mitochondrial permeability transition (MPT), which is associated with loss of mitochondrial energy, mitochondrial matrix inflammation, and external membrane rupture; this is
accompanied by the release of numerous proapoptotic factors; in addition, TNF-α activates different hepatic cell cascades, resulting in the stimulation of mitotic genes such as p38 of Mitogen-activated protein kinases (MAPK) and the Jun N-terminal kinase (JNK), which affect mitochondrial sensitivity to the proapoptotic. Acute ethanol consumption can produce a hypermetabolic state in the liver that is characterized by increase in mitochondrial respiration, which is driven by the great demand for NADH reoxidation produced during ethanol metabolism by cytosolic ADH (Adachi and Ishii, 2002); in addition, it alters hepatic microcirculation by stimulating endothelial-1 production (Thurman, 1998); similarly, the acetaldehyde generated by ethanol metabolism causes hypoxia on chemically reacting with free sulfate groups such as glutathione, in such a way as to alters the reaction of this metabolite, which activates the xantine oxidase and xantine dehydrogenase enzymes, in order to finally diminish the NAD⁺/NADH equilibrium (Gutierrez-Salinas and Morales-Gonzalez, 2004). Depletion of antioxidant levels, above all that of hepatic glutathione, caused by acute as well as by chronic ethanol consumption, increases oxidative stress, which induces changes in the mitochondrial membrane, such as diminution of the mitochondrial membrane potential in hepatocytes and MPT, both inhibited by the antioxidants or by an ADH inhibitor (Adachi and Ishii 2002).

7.1.7 Inhibition of hepatic regeneration by ethanol: When there is a significant loss in functional hepatic mass, such as in partial hepatoctomy, the remnant tissue undergoes a regeneration process in which the removed tissue is replaced in its totality; during this process, DNA synthesis increases notably in post surgery. After partial hepatoctomy, hepatic tissue become more vulnerable to the damage caused by consumption of xenobiotics, particularly ethanol administration, which affects hepatic regeneration in the early regenerative process phase (Morales-Gonzalez et al., 2001). Studies performed on animals suggest that the acute ethanol administration rapidly inhibits the result of the hepatic regeneration after surgery. Although the exact mechanism by which ethanol inhibits hepatic regeneration is not clear, it is reasonable that hepatotoxicity could alter the total metabolism of the regenerating liver, which includes ethanol oxidation into acetaldehyde, catalyzed by ADH, and the later conversion of this into acetate by means
of the mitochondrial ALDH (Gutierrez-Salinas, 1999). Acute ethanol administration produces structural and biochemical changes such as partial inhibition of protein and DNA synthesis, which indicates the diminution of the mitotic index, transitory accumulation of fat, the presence of inflammation, modifications in hepatocellular organization, diminution of weight gain in the regenerating liver, and inhibition of hepatic regeneration (Morales-Gonzalez et al., 1999, 2001). Some physiological processes that are altered by ethanol are in serum (glucose, triglycerides, albumin, and bilirubin), in addition causing modification of the serum activity of enzymes that reflect liver integrity (alanine and aspartate aminotransferase, lactate dehydrogenase, ornithine carbamoyltransferase, and glutamate dehydrogenase); also, on inhibiting DNA synthesis and the activity of enzymes intimately related with this process, such as Thymidine synthetase (TS) and Thymidine kinase (TK), in addition to diminution of the mitotic index (Morales-Gonzalez et al., 2001). A sole dose of ethanol is capable of significantly inhibiting the synthesis of the protein ornithine decarboxylase, in addition to causing thyrosine aminotransferase degradation, which suggests that acute ethanol consumption inhibits protein synthesis and regenerating liver activity on transcriptional levels, interfering with RNA synthesis in the nucleus (Morales-Gonzalez et al., 2001). Investigations demonstrates that the acute as well as the chronic ethanol administration jeopardize the incorporation of thymidine into the DNA of hepatocytes of rats on which partial hepatoctamy had been performed with or without diminution of the DNA contents, in addition to reporting that chronic consumption of this substance inhibits regeneration 24 hours after the partial hepatoctamy due to delay in the induction of ornithine carbamoyltransferase activity (Yoshida et al., 1997). It has been suggested that damage caused by free radicals as the product of ethanol consumption occurs at the early phase of hepatic regeneration; on the other hand, a transcending increase has been reported in mitochondrial lipoperoxidation of the liver in rats after partial hepatoctamy. In the same study, a diminution was also observed in the early hepatic regeneration phase of mitochondrial glutathione levels (Guerrieri et al., 1998).

7.1.8 Ethanol and free radicals: A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital.
The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. Examples of oxygen free radicals are superoxide (O$_2^*$), hydroxyl (OH$^*$), peroxyl (RO$_2^*$), alkoxyl (RO$^*$), and hydroperoxyl (HO$_2^*$) radicals. Nitric oxide and nitrogen dioxide ($^*$NO$_2$) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCI), hypobromous acid (HOBr), and peroxynitrite (ONO$_2^-$). ROS, reactive nitrogen species (RNS), and reactive chlorine species are produced in animals and humans under physiologic and pathologic conditions and are included radical and non-radical species. These radicals attack molecules like fat, proteins, DNA, sugar etc., the newly damaged molecule unfortunately becomes a free radical and thus a chain reaction is started. Free radicals are generated during normal metabolism and exposure to environmental insults such as infections agents, pollution, UV light, radiation and so on. These ROS are cellular renegades and wreak havoc in biological system by tissues damage, altering biochemical compounds, corroding cell membranes and killing out rightly (Wiseman and Halliwell, 1996). These are highly reactive species capable of wide-spread, indiscriminate oxidation and peroxidation of proteins, lipids and DNA which can lead to significant cellular damage and even tissue and/or organ failure.

Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on the organisms. For example, oxygen radicals exert critical actions such as signal transduction, gene transcription, and regulation of soluble guanylate cyclase activity in cells. Also, NO is one of the most widespread signaling molecules and participates in virtually every cellular and organ function in the body (Ignarro et al., 1999). Physiologic levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone, and hemodynamics (Ignarro et al., 1999). In addition, NO produced by neurons serves as a neurotransmitter, and NO generated by activated
macrophages is an important mediator of the immune response (Freidovich, 1999). However, as oxidants and inhibitors of enzymes containing an iron-sulfur center, free radicals and other reactive species cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA), which leads to cell injury and death. For example, radiation-induced ROS markedly alter the physical, chemical, and immunologic properties of SOD, which further exacerbates oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system. Thus, there are “two faces” of free radicals in biology in that they serve as signaling and regulatory molecules at physiologic levels but as highly deleterious and cytotoxic oxidants at pathologic levels. One factor that suggested that ethanol causes cell damage due to its hepatic metabolism is the excessive generation of free radicals, which can be the result of a state denominated oxidative stress; this is because any of ethanol’s metabolic pathways, principally MEOS, is made up of chemicals oxido-reduction reactions, which produce highly unstable molecules called Reactive oxygen species (ROS), such as the superoxide anion (O$_2^-$), Hydrogen peroxide (H$_2$O$_2$), and the hydroxyl radical (OH$^-$) (Nanji and French, 2003). Free radicals can perform four main reactions (Wu and Cederbaum, 2003):

1. Hydrogen abstraction, in which free radicals interact with another molecule that acts as the donor of an atom of H+. As a result, free radicals bind to H+ and become more stable, while the donor is converted into free radicals.

2. Addiction. Because the free radicals binds to a more stable molecule, which converts a receiver molecule into free radical.

3. Termination. In this, two free radicals react between themselves to form a more stable compound.

4. Disproportion. This consists of two lone radicals that are identical to each other react between them. In this reaction, one free radical acts as an electron donor and the other, as an electron receiver. In this manner, they become two more stable molecules. Free radicals are chemical species that possess an unpaired
electron in their last layer, which allows these to react with a high number of molecules of all types, first oxidizing these and afterward attacking their structures. If lipids (polyunsaturated fatty acids) are involved, they damage the structures rich in the latter, such as cell membranes and lipoproteins (Rodríguez et al., 2001). Within this generic concept, the partially reduced forms of oxygen are denominated Reactive oxygen species (ROS). This is a collective term that includes not only oxygen free radicals, but also some reactive non-radical oxygen derivatives. The oxidizing mechanism of free radical is intimately linked to their origin, which follows a sequence of chain reactions; in these reactions, a very reactive molecule is capable of reacting with another, non-reactive molecule, inducing in the latter the formation of a free radical ready to initiate a new neutrophilic attack, and so on successively.

![Diagram showing production of free radicals in different parts of the body and their deleterious diseases.](http://qvhealthwealthprosper.com/tag/free-radicals/)

The greatest source of ROS production in the cell is the mitochondrial respiratory chain, which utilizes approximately 80-90% of the $O_2$ that a person consumes; another important source of ROS, especially in the liver, is a group of Mixed function oxidase (MFO) Cytochrome P-450 enzymes. In addition to the ROS generation that takes place naturally in the organism (Figure 6.3), humans are constantly exposed to environmental
free radicals including ROS in the form of radiation, Ultraviolet (U.V) light, smoke, tobacco smoke, pesticides, and drugs utilized in the treatment of cancer. In addition, intake of alcohol results in excessive generation of free radicals, which alter the biomembranes and cause damage. To scavenge ROS, cell have several antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione-Stranferase (GST) (Wu and Cederbaum, 2003). The increase in $O_2^-$ and $H_2O_2$ formation is justified with the finding that in aging, electron flow conditions are modified in the transport chain of these, which is the last stage of high energy proton production, and whose passage through the internal mitochondrial membrane generates an electrical gradient that provides the energy necessary for forming ATP. Researchers postulate that the ROS generated can produce damage to the internal mitochondrial membrane as well as to electron transport chain components or to mitochondrial DNA, which further increases ROS production and consequently, more damage to the mitochondria and an increase of oxidative stress due to increased oxidant production. Free radicals produced during aerobic stress cause oxidative damage that accumulates and results in a gradual loss of the homeostatic mechanisms, in an interference of genetic expression patterns, and loss of cell functional capacity, which leads to aging and death. ROS generation promotes the decrease of intracellular glutathione, elevation of cytoplasmic calcium, lipid peroxidation of the membranes, and a series of chain reactions that are accompanied by the disappearance of glycogen, decrease of ATP, and the descent of the energy state of hepatic cells; these events are the origin of membrane destruction and cell death (Thurman et al., 1999). Sustained elevation of cytoplasmic calcium is associated with activation of calcium-dependent enzymes such as phospholipase A2, glycogen phosphorylase, and the endonucleases, which cause plasmatic membrane ruptures and DNA molecule fragmentation. On the other hand, the transitory elevation of intracellular calcium intervenes in the progression of cell division in G1 to S transitions and in those of the G2 to M phase. Immediately after cell death, hepatocellular proliferation begins in order to reestablish cell populations that have been destroyed, thus restoring hepatic function (Andres and Cascales, 2002). The OH$^-$ radical is highly toxic for the hepatocytes, which do not possess a direct system for its elimination; this free radical is produced intracellularly.
by two reactions that occur spontaneously and that are catalyzed by a transition metal, generally iron (Fe), which are termed the Fenton reaction and the Haber-Weiss reaction (Boveris et al., 2000). In this reaction, Hydrogen peroxide (H$_2$O$_2$) in the presence of Fe as catalyst produce the hydroxyl radical (OH$^\cdot$). The superoxide radical (O$_2^-$) leads to the formation of Hydrogen peroxide (H$_2$O$_2$) and both products of the partial reduction of oxygen (O$_2$) produce the hydroxyl radical (OH$^\cdot$).

7.2 REVIEW OF LITERATURE

The conversion of grape juice to wine is a biotechnological tradition dating back to the dawn of civilization. Throughout the ages numerous wine-making strategies were developed resulting in the range of wine products, from champagne to port, available today. However, since the time of Louis Pasteur (1873) the microbial contribution to the production of wine has become a subject of research, and often a debate. Wine composition and quality are functions of many different intrinsic and extrinsic variables, many of which are microbiologically mediated. A large diversity of microbes are inherent to wine-making including various yeasts, bacteria and fungi. Prominent in this process are Saccharomyces cerevisiae, which dominate the alcoholic fermentation, and the lactic acid bacteria (LAB), which carry out the malolactic conversion.

Through the 1990s, a remarkably consistent epidemiological data has accumulated pointing to the reduced incidence of mortality and morbidity from coronary heart disease (CHD) among those who consume alcohol in moderation by comparison with abstainers. This reduction has been demonstrated in diverse populations, in both sexes, and all ages. This protection seems in large measure due to the ethanol present in those beverages classified as “alcoholic,” but there is some evidence that wine confers additional benefits, due to its content of polyphenols, which at least in vitro and in cell culture experiments, act as potent inhibitors of platelet aggregation, eicosanoid synthesis, and biological oxidation reactions associated with the generation of free radicals. A major advantage of wine as a matrix for dietary polyphenols is that they are soluble and bioavailable in wine, in contrast to vegetable foodstuffs, which contain their phenolic components in polymeric, insoluble or tightly
bound, and compartmentalized forms that render them unavailable for absorption. Awe and Olayinka, (2011) did not found any disruptions in liver structure histologically or enzyme activities assessment after treatment with cashew wine (5% alcohol); however till now probably there was no report on assessment of in vivo antioxidant activity of non-grape fruit wine.

In recent years, in view of various health benefits and health promoting affects, wine became a popular beverage and the imposed legal restrictions were called off in some countries including India. Ministry of food processing industries (MoFPI), Govt. of India, has been encouraging alcoholic beverages from non-molasses sources from past many years. Hence various research teams in India have focused on production and development of wine from non grape source as grapes are not much cultivated in India and may not be feasible economically. A research group headed by Prof. V.K. Joshi from Department of Postharvest Technology, Dr. Y.S Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh has focused on production of wine from locally available berries, plums, cherries, currants, peaches, strawberries and from other fruits. However from South India, not much extensive work on wine was taken up, except few reports from Central Food Technological Research Institute (CFTRI), Mysore, India. Hence initially, Reddy and Reddy (2005) has screened seven different mango cultivars and found that physic chemical characteristics and volatile profile of mango wine was similar to red wine. Several reports were available on various developments on mango wine (Reddy and Reddy, 2009, Kumar et al., 2009, Reddy et al., 2010a, Li et al., 2011).Kumar et al., (2011) has reported in vitro antioxidant activity of mango wine from different cultivars. Hence this study was aimed to assess the in vivo antioxidant activity of mango wine in rat model system.

7.3 MATERIALS AND METHODS

7.3.1. In vitro antioxidant activities of mango wine

7.3.1.1. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity: Diluted mango wine (1:10) of different volumes (20, 40, 60, 80 and 100 μL) were taken, and the
alcohol was evaporated under nitrogen flush. Mango wine was assayed for radical scavenging activity by the procedure of Duh and Yen (1997). The radical scavenging activity of the extract is expressed as a percentage decrease in the absorbance of DPPH against that of the blank at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity = (control OD - sample OD/control OD) × 100.

7.3.1.2 Antioxidant activity on rat low density lipoprotein (LDL) oxidation: Plasma was separated from blood drawn from male albino (Wistar) rats and stored at 4°C until used. The isolation of LDL from the plasma and measurement of the antioxidant activity of the diluted (1:10) mango wine (20, 60 and 100 µL) were done according to the method of Princen et al. (1992) at 2, 4, and 6 h intervals. The percentage protection was calculated using the following formula: (oxidation in control - oxidation in experimental/oxidation in control) × 100.

7.3.2 Animals and treatments: Experiments were carried out with male albino rats of Wister strain, weighing 180±50 g. They were obtained from Sri Raghavendra suppliers, Bangalore and maintained in standard laboratory conditions, with a 12 hr light/dark cycle and fed with commercial rat feed supplied by Hindustan Lever Ltd., Mumbai under the trade name Gold Mohur rat feed and water ad libitum. Experimental animals were handled according to the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Animal Ethical Committee, Sri Venkateswara University (Resolution No. 1/(i)/a/CPCSEA/IAEC/SVU/OVS-SVK/Dt.27-01-2011). At 6 months of age, 30 animals were randomly divided into five experimental groups of 6 animals each and treated as follows and the present study design was according to Arola et al., (1997).

1. **Pair-fed control (PFC) group.** Rats were given the same amount of pellet diet as the EtOH group. The calories provided by ethanol were replaced by an isocaloric amount of sucrose, considering that 1 g of sucrose corresponds to 4 kcal and 1 g of ethanol to 7 kcal, and that the density of ethanol is 0.79 g/mL.
II. *Ethanol (EtOH)* group. Animals were treated with an aqueous ethanol solution as the only available liquid source, starting with a 5% (v/v) ethanol solution and increased progressively by 1% per day to a final concentration of 12% (v/v) 2 weeks later. These rats had free access to standard pellet diet.

III. *Mango wine (MW)* group. Animals were given mango wine containing 12% (v/v) ethanol as the only available drinking fluid. The introduction of mango wine was gradual, starting with a 5% ethanol (v/v) solution similar to EtOH treated rats. Animals had also free access to standard pellet diet.

IV. *Dealcoholised mango wine (DMW)* group. As a liquid source these rats had free access to a Dealcoholised (alcohol free) mango wine as the only available drinking fluid and were given the same amount of standard pellet diet.

V. *Red wine (RW)* group. Rats had free access to red wine as the sole drinking fluid in which ethanol concentration was also increased to 12% (v/v) to equalize the ethanol content in beverages ingested by the EtOH and MW groups and animals had unrestricted access to standard pellet diet.

7.3.3 Blood and tissue analyses: At the end of experimental period, the animals were fasted for 12h to minimize dietary changes and sacrificed by cervical dislocation. Immediately blood was collected in to sterile tubes by cardiac puncture and serum was separated from the cells by centrifugation at 3000rpm for 10 min. The tissues (liver and kidney) were removed and washed thoroughly with ice-cold saline (0.9% NaCl) and processed appropriately, stored at -20°C in propylene containers until assays were carried out.

7.3.3.1 Preparation of the tissue homogenate: Samples of liver and kidney (100 mg/mL) were homogenized in 50 mM Tris-Hcl buffer (pH 7.0), and then centrifuged at 10,000 rpm for 15 min; the supernatant thus obtained was used for assaying the biochemical parameters.
7.3.3.2 Histopathological studies: The tissues (liver and kidney) preserved in neutral buffered formalin were used for the study of histopathological changes. Tissues were processed, which involves dehydration, clearing and infiltration of the tissue with paraffin and then the tissues were sectioned. The sections are mounted on glass slides and smeared with a drop of Mayer’s egg albumin. The slides are dried on a hot plate at about 50°C for 30 min. The sections are then stained with Mayer’s Hematoxylin-Eosin stain and observed under light microscope.

Estimation of protein: The estimation of protein was carried out by the method of Lowry et al. (1951).

Reagents:

1. Bovine serum albumin (BSA) was used as a standard (0.1 mg/mL).
2. Sodium carbonate (Na₂CO₃), 4% in 0.1 N Sodium hydroxide (NaOH);
3. Sodium potassium tartrate: 2%
4. Copper sulphate: 1%
5. Lowry alkaline copper solution: Reagents 2, 3 and 4 are mixed in the proportions of 100:1:1 just before use.
6. Folin’s phenol reagent: Commercially available Folin ciocalteau’s reagent was diluted with distilled water in 1:1 proportion.

Procedure: 25 μL of the tissue homogenate was made up to 1 ml with distilled water and to this added 5 mL of alkaline copper reagent and the contents were kept at room temperature (RT) for 20 min. After the incubation, to the test tubes added 0.5 ml of Folin ciocalteau’s reagent and the tubes were kept at RT for 10 min. The colour developed was read at 650 nm. Blank and standards were also run simultaneously.

7.3.3.3 Estimation of bilirubin: Bilirubin content was estimated according to the procedure of Malloy and Evelyn, (1937).
Reagents:

1. Absolute alcohol
2. Hydrochloric acid, 1.5% v/v in distilled water
3. Diazo-reagent, prepared by adding 0.3 mL of solution B to 10 mL of solution A.
4. Solution A: 1g of sulphanilic acid was dissolved in 15 mL of concentrated HCl and made up to 1 litre with distilled water.
5. Solution B: 0.5 g of sodium nitrite was dissolved in water and made up to 100 mL.
6. Standard solution of bilirubin-A solution containing 10 mg bilirubin per 100 mL of chloroform was prepared.

Procedure: Two test tubes were taken and into each 0.2 mL of serum and 1.8 mL of distilled water were added. To the unknown 0.5 mL of diazo reagent was added and to the blank 0.5 mL of 1.5% HCl. Finally to each 2.5 mL of methanol was added. Allowed to stand for 30 minutes and the optical density was read in a colorimeter set at 540 nm. Calibration curve was constructed simultaneously with the standards. The amount of bilirubin was calculated by extrapolating O.D values using a standard curve.

7.3.3.4 Estimation of urea: Blood urea nitrogen was estimated by the method of Wybenga et al., (1971) by measuring the coloured complex formed with diacetyl monoxime in acidic medium.

Reagents:

1. Sodium tungstate, 10%: 10 g of sodium tungstate was weighed and dissolved in 100 mL of distilled water.
2. 2/3 N sulphuric acid.
3. Diacetyl monoxime (2%) in 2% acetic acid.
4. Sulphuric acid-phosphoric acid reagent: 140 mL of water was mixed with 150 mL 75% phosphoric acid and then 50 mL of concentrated sulphuric acid was added slowly.
5. Standard: 250 mg of urea was dissolved in 100 mL of distilled water. This solution was diluted 1: 100 to give a solution containing 25μg/mL, which was used as the working standard.

Procedure: 0.1 mL of serum sample was mixed with 3.3 mL of distilled water, 0.3 mL of sodium tungstate and sulphuric acid, mixed well and centrifuged. 1 mL of supernatant was mixed with 1 mL of distilled water, 0.4 mL of diacetyl monoxime and 1.6 mL of sulphuric acid-phosphoric acid reagent. The tubes were placed in a boiling water bath for 30 min and cooled. The colour developed was read at 480 nm in a colorimeter against water as blank. The same procedure was performed with a series of standards. Serum urea level is expressed as mg/dL of serum.

7.3.3.5 Estimation of uric acid: Serum uric acid was estimated by the method of Caraway (1963).

Reagents:
1. Phosphotungstic acid: To prepare a stock solution, 50 g of sodium tungstate (Na₂WO₄·2H₂O) was dissolved in 400 mL of distilled water. 40 mL of 85% phosphotungstic acid was refluxed gently for 2 hr, cooled and transferred to a 500 mL flask and made up to the mark with distilled water. This reagent was diluted 1: 10 and can be stored in a brown bottle for months.
2. Sodium carbonate: 15%
3. Standard: 100 mg of uric acid and 60 mg of lithium carbonate were dissolved in about 50 mL of distilled water. This was heated to about 60°C to dissolve the uric acid completely. After cooling, the solution was finally made up to 100 mL with distilled water. 1 mL of stock standard was diluted to 10 mL to give a working standard concentration of 10μg/mL.

Procedure: About 0.5 mL of serum was taken and to this 2.5 mL of distilled water was added followed by 0.6 mL of phosphotungstic acid and 0.6 mL of sodium carbonate. A blank was setup with 3 mL of distilled water. Standard with graded volumes were also
setup. After 10 min, the colour developed was read at 640 nm in a colorimeter. Uric acid level is expressed as mg/dL.

7.3.3.6 Estimation of creatinine: Serum creatinine was estimated by the method of Owen et al., (1954).

Reagents:
1. Picric acid, 0.04 M: dissolve 916mg of picric acid in 100 mL of distilled water.
2. Sodium hydroxide, 0.75 N was prepared in distilled water.
3. Standard: 100 mg of creatinine was dissolved in 0.1 N Hydrochloric acid and made up to 100 mL. 1 mL of this solution was diluted to 10 mL with distilled water to give a working standard containing 100 µg/mL.

Procedure: 0.1 mL of serum sample was made up to 2 mL with distilled water. Standards in the range of 10-40 µg/mL were also made up to 2 mL. Blank contained only distilled water. To all the tubes, 1 mL of picric acid followed by 1 mL of sodium hydroxide was added. The resulting colour was read at 540 nm after 15 min. Creatinine level is expressed as mg/dL.

7.3.3.7 Estimation of triglycerides: Serum triglycerides were measured by the method of Foster and Dunn (1973).

Reagents
1. Standard triolein in isopropanol was used as standard (0.1 mg/ml).
2. Activated alumina (neutral)
3. Isopropanol
4. Alcoholic KOH: 50 g of KOH in 1 litre of mixture of iso-propanol and water (2:3)
5. Acetyl Acetone reagent: To 200 ml of Isopropanol added 7.5 ml of acetyl acetone and the contents were made up to 1 litre with distilled water.
6. Sodium meta-periodate: 60 ml of acetic acid and 77g of anhydrous ammonium acetate were added to 700 ml of water. 650 mg of sodium meta
periodate was dissolved in this solution and the final volume was made up to 1 litre with distilled water.

**Procedure:** 0.1 ml of serum was taken in a glass stoppered centrifuge tube and to this added 4 ml of isopropanol and 400 mg of alumina. The tubes were tightly capped and shaken vigorously for 10 min. Then the tubes were centrifuged at 3000 rpm for 15 min and 2 ml of the supernatant was pipetted into clean, dry test tubes. To these added 0.6 ml of alcoholic KOH and kept at 70°C for 15 min. The tubes were cooled to RT. To this added 0.5 ml of acetyl acetone reagent, 1.0 ml of meta periodate reagent and incubated at 50°C for 30 min. Standard was also run in the same fashion with triolein instead of serum. The colour developed was read at 405 nm against the reagent blank.

7.3.3.8 Estimation of HDL-cholesterol (HDL-C): Determination of serum HDL-cholesterol was carried out by the method of Burstein *et al* (1970).

**Reagents**

1. Phosphotungstic acid reagent: To 200 ml of distilled water added 22.5 g of phosphotungstic acid and 80 ml of 1M sodium hydroxide and the volume was made up to 500ml with distilled water.

2. Magnesium chloride solution: 101.7g of MgCl₂ was dissolved in 250 ml of distilled water.

**Procedure:** 0.5 ml of serum was taken in a centrifuge tube and to this added 0.25 ml of phosphotungstic acid reagent and 0.25 ml of MgCl₂ and was centrifuged at 1500 × g for 30 min in a refrigerated centrifuge and the amount of cholesterol was determined.

7.3.3.9 Estimation of VLDL and LDL cholesterol (VLDL-C and LDL-C): By using Freidwald formula the concentration of VLDL and LDL cholesterol in serum were calculated (Friedwald *et al*., 1972).

\[
VLDL-C = \frac{\text{Triglycerides}}{5}
\]

LDL-C= (Total Cholesterol – VLDL-C) - (HDL-C)
7.3.3.10 Estimation of cholesterol: Cholesterol content was estimated by the method of Parekh and Jung (1970).

Reagents:

1. Ferric chloride-uranyl acetate reagent: 500 mg of ferric chloride was dissolved in 10 mL of distilled water; 3 mL of concentrated ammonia was added to it and centrifuged. The precipitate was washed several times with distilled water and dissolved in one liter of glacial acetic acid. 100 mg of uranyl acetate was added to the mixture and the contents were shaken well and kept over night. The reagent was also stable for six months. Sulphuric acid-ferrous sulphate reagent: 100 mg of ferrous sulphate was dissolved in 100 mL of glacial acetic acid and 100 mL of sulphuric acid. After cooling to room temperature, the volume was made up to one liter with concentrated sulphuric acid and stored in a brown bottle.

2. Cholesterol standard: 200 mg of cholesterol (recrystallised from ethanol) was dissolved in 100 mL of chloroform. 1 mL of stock cholesterol was diluted to 100 mL to obtain a working standard having 20µg cholesterol/mL.

Procedure: To 0.1 mL of serum, 3 mL of ferric chloride-uranyl acetate reagent was added. Then 2 mL of sulphuric acid-ferrous sulphate reagent was added to all the tubes and the contents were mixed well. After 20 min the colour developed was read against reagent blank at 540 nm. Standards were also treated in a similar manner. Serum cholesterol content is expressed as mg/dL.

7.3.3.11 Estimation of blood glucose

Blood glucose was estimated by the modified method of Sasaki and Matsui (1972) using O-toluidine reagent.

Reagents:

1. Trichloroacetic acid: 10% solution

2. Ortho-toluidine reagent: 12.5 g of thiourea and 12.0 g of boric acid were dissolved in 50 mL of distilled water by heating over a mild flame. 75 mL of
redistilled ortho-toluidine and 375 mL of acetic acid were mixed separately. The two solutions were mixed and the total volume was made up to 500 mL with distilled water. The reagent was left in a refrigerator overnight and filtered.

3. Standard glucose: 10 mg of anhydrous glucose was dissolved in 100 mL of water containing 0.01% benzoic acid as preservative to give a concentration of 100 μg/mL.

**Procedure:** 0.1 mL of blood was immediately mixed with 1.9 mL of trichloroacetic acid solution to precipitate proteins and then centrifuged. 0.1 mL of supernatant was mixed with 4.0 mL of O-toluidine-boric acid reagent and was kept in a boiling water bath for 15 minutes along with blank. The green colour developed was read at 600 nm. A standard curve was obtained using standard glucose solution in the concentration of 20-100 μg/mL. The values were expressed as mg/dL of blood.

7.3.3.12 Estimation of serum glutamate oxaloacetate transaminase/aspartate amino transferase (SGOT/AST) activity: SGOT/AST was assayed by the method of King (1965a). The reaction catalyzed by this enzyme involves the formation of glutamate and oxaloacetate from the substrate containing aspartic acid and 2-oxoglutaric acid. The oxaloacetate thus formed was allowed to react with DNPH reagent. The colour developed was measured at 540 nm after the addition of NaOH. A set of standard pyruvate was also run in a similar manner.

**Reagents:**

1. Phosphate buffer, 0.1 M, pH 7.5.
2. Substrate: 1.33 g weight of aspartic acid and 1.5 mg of 2-oxoglutarate were dissolved in 20.5 mL of 1N NaOH and made up to 100 mL with distilled water.
3. 2,4-dinitro phenyl hydrazine (DNPH) 0.002%: 20 mg of DNPH was dissolved in 100 mL of 1N HCl.
4. Sodium hydroxide, 0.4 N.
5. Standard: 11 mg of sodium pyruvate was dissolved in 100 mL of phosphate buffer (pH 7.5). This contains 1μmol pyruvate/mL.
**Procedure:** 1 mL of substrate was incubated at 37°C for 10 min. Then, 0.2 mL of enzyme was added and the mixture was incubated at 37°C for 1 hr. To the control tubes the enzyme was added after the reaction, and it was arrested by the addition of 1 mL of DNPH reagent. The tubes were kept at RT for 30 min. Then 5 mL of sodium hydroxide was added. A set of standard pyruvate solution were also treated in a similar manner. The color developed was read against the blank at 540 nm using a colorimeter. The enzyme activity was expressed as units/L (One unit corresponds to enzyme that liberates one μM of pyruvate/min).

### 7.3.3.13 Estimation of serum glutamate pyruvate transaminase/alanine aminotransferase (SGPT/ALT) activity:

SGPT was assayed by the method of King (1965a). This enzyme catalyzes the formation of pyruvate and glutamate from alanine and 2-oxoglutaric acid. The pyruvate formed was made to react with DNPH reagent and the colour developed was measured at 540 nm after the addition of NaOH. A set of standard pyruvate was also run simultaneously.

**Reagents:**

1. Phosphate buffer, 0.1 M, pH 7.5.
2. Substrate: 1.78 g of DL-alanine and 30 mg of 2-oxoglutarate were dissolved in 20 mL of phosphate buffer (pH 7.5). 0.5 mL of 1N NaOH was added and made up to 100 mL with distilled water.
3. 2, 4-dinitro phenyl hydrazine (DNPH) 0.002%: 20 mg of DNPH was dissolved in 100 mL of 1N HCl.
4. Sodium hydroxide, 0.4 N.
5. Standard: 11 mg of sodium pyruvate was dissolved in 100 mL of phosphate buffer (pH 7.5). This contains 1μmol pyruvate/mL.

**Procedure:** 1 mL of substrate was incubated at 37°C for 10 min. Then, 0.2 mL of enzyme was added and the mixture was incubated at 37°C for 30 min. To the control tubes the enzyme was added after the reaction, and it was arrested by the addition of 1 mL of DNPH reagent. The tubes were kept at room temperature for 20 min. Then 5 mL of sodium hydroxide was added. A set of standard pyruvate solution were also treated in
a similar manner. The color developed was read at 540 nm using a colorimeter. The enzyme activity was expressed as units/L (One unit corresponds to enzyme that liberates one μM of pyruvate/min).

7.3.3.14 Estimation of alkaline phosphatase (ALP) activity: The activity of alkaline phosphatase was assayed by the method of King (1965b) using disodium phenyl phosphate as substrate. The colour developed is read at 640 nm.

Reagents:

1. Carbonate-bicarbonate buffer, 0.1 M, pH 9.8.
2. Disodium phenyl phosphate, 0.1 M: 218 mg of disodium phenyl phosphate was dissolved in 100 mL of distilled water.
3. Magnesium chloride, 0.1 M: 952.2 mg of magnesium chloride was dissolved in 100 mL of distilled water.
5. Standard: 10 mg of phenol was dissolved in 100 mL of distilled water. The standard solution contained 100 μg/mL.

Procedure: The incubation mixture containing 1.5 mL buffer, 1 mL substrate, 0.1 mL magnesium chloride were preincubated at 37°C for 10 min. Then 0.1 mL of enzyme was added and incubated at 37°C for the 15 min. The reaction was arrested by the addition of 1 mL of Folin-phenol reagent. The control tubes received enzyme after the addition of Folin -phenol reagent. Then 1 mL of sodium carbonate was added and the tubes were incubated at 37°C for 10 min. The colour developed was read at 640 nm in a colorimeter. Standards and blank were treated in a similar manner. The activity is expressed in terms of units/L (One unit corresponds to enzyme that liberates one μM of phenol/min/mg protein under incubation conditions).

7.3.3.15 Estimation of γ-glutamyl transpeptidase activity, (E.C.2.3.2.2): The activity of γ-glutamyl transpeptidase was estimated according to the method modified by Rosalki and Rau (1972). γ-glutamyl transpeptidase catalyses the transfer of the γ-
glutamyl group from \( \gamma \)-glutamyl peptides to another peptide or the L-amino acid or the removal of the glutamyl group from some compounds which contain it.

**Reagents:**

1. Tris-HCl buffer, 0.1 M, pH 8.5
2. Glycylglycine: 13.2 mg/10 mL.
3. Substrate: 30.37 mg of L- \( \gamma \)-glutamyl-p-nitroanilide was dissolved in 10 mL of water by heating at 50-60°C.
4. Standard: 13.8 mg of p-nitroaniline was dissolved in 100 mL of distilled water. This contained 1 \( \mu \)mol/mL.

**Procedure:** The incubation mixture contained 0.5 mL of substrate, 1 mL of Tris-HCl buffer, 2.2 mL of glycylglycine, 0.2 mL of enzyme was added to the above mixture and the total volume was made up to 4 mL with water. After incubation for 30 min at 37°C, the samples were heated at 100°C for 5 min and centrifuged. The amount of p-nitroaniline in the supernatant was measured at 410 nm. The activity of \( \gamma \)-glutamyl transpeptidase was expressed as units/L.

7.3.3.16 Estimation of lactate dehydrogenase activity, (EC 1.1.1.27): Lactate dehydrogenase activity was determined by the method of King. (1965).

**Reagents:**

1. 0.1 M glycine buffer (pH 7.6): 7.5 g of glycine and 5.85 g of sodium chloride was dissolved in one liter of distilled water.
2. Buffered substrate: 2.76 g of lithium lactate was dissolved in 1.25 mL of glycine buffer containing 75 mL of 0.1 N NaOH solutions. This was prepared just before use.
3. 0.4 N NaOH solution
4. 5.0 mg of NAD\(^+\) was dissolved in 10 mL of distilled water (Prepared freshly every time).
5. 2, 4-dinitrophenylhydrazine (DNPH): 200 mg of DNPH was dissolved in 1 L of 1N HCl.
6. Standard pyruvate solution: 12.5 g of sodium pyruvate was dissolved in 100 mL of buffered substrate solution.

**Procedure:** To 1.0 mL of the buffered substrate, 0.1 mL of the sample was added and the tubes were incubated at 37°C for 15 min. After adding 0.2 mL of NAD⁺ solution, the incubation was continued for another 15 min. The reaction was arrested by adding 1.0 mL of DNPH reagent. The tubes were then incubated for a further period of 15 min after which 7 mL of 0.4 N NaOH was added and colour developed was measured at 420 nm using a spectrophotometer. The calibration curve was constructed simultaneously with the test sample. LDH activity was expressed as units/L (one unit corresponds to 1 μM of pyruvate formed/min).

7.3.3.17 Estimation of lipid peroxidation in tissues: Lipid peroxidation in tissues was carried out by the method of Okhawa et al., (1979). Estimation of lipid peroxides was based on the reduction of thiobarbituric acid to give a pink coloured complex, MDA which is measured at 532 nm. MDA formed as an end product of the peroxidation of lipids served as an index of the intensity of oxidative stress.

**Reagents:**
1. 1.15% KCl.
2. 8.1% Sodium dodecyl sulphate (SDS)
3. 0.8% Thiobarbituric acid.
4. n-butanol: pyridine mixture (15:1 v/v).
5. Standard: 1, 1, 2, 2-tetraethoxy propane (TEP)

**Procedure:** The assay mixture contained 0.1 mL of 10% tissue homogenate (prepared in 1.15% KCl), 0.2 mL of SDS and 1.5 mL of TBA. The mixture was finally made up to 4 mL with distilled water and boiled at 95°C for 1 hr. After cooling, 1 mL of distilled water and 5 mL of n-butanol: pyridine mixture were added and shaken vigorously and then centrifuged at 4000 rpm for 10 min. Then the absorbance of the organic layer was measured at 532 nm. Amount of lipid peroxidation is expressed as nano moles of MDA.

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produced/mg protein. A standard curve of TEP was prepared in a similar condition and used for calculation.

7.3.4 Enzymic antioxidants

7.3.4.1 Estimation of superoxide dismutase activity, (E.C.1.15.1.1): Superoxide dismutase was measured according to the method of Misra and Fridovich, (1972) based on the oxidation of epinephrine transition by the enzyme.

Reagents:

1. 50 mM Sodium carbonate-bicarbonate buffer, pH 10.2 containing 0.1 mM of ethylene diamine tetra acetic acid (EDTA).
2. 0.6 mM epinephrine (Adrenaline).

Procedure: To 0.5 mL of the homogenate 2 mL of carbonate buffer and 0.5 mL of 0.6 mM epinephrine was added. Epinephrine was the last component to be added and the adrenochrome formed in the next 4 min was recorded at 470 nm in spectrophotometer. SOD activity is expressed in units/min/mg protein (One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of epinephrine auto-oxidation).

7.3.4.2 Estimation of catalase activity, (E.C.1.11.1.6): Catalase assay was carried out by the method of Aebi, (1984). The decomposition of H₂O₂ was followed directly by measuring the decrease in absorbance at 240 nm.

Reagents:

1. 50 mM Phosphate buffer, pH 7.0
2. 30 mM H₂O₂: 340 μL of 30% (v/v) H₂O₂ was dissolved in 100 mL of phosphate buffer (pH 7.0).

Procedure: 0.5 mL of the tissue homogenate was mixed with 1.5 mL of phosphate buffer. Then 1 mL of H₂O₂ was added and change in absorbance was recorded after
every 15 sec up to 1 min. The activity was expressed as μmoles of H₂O₂ utilized/min/mg protein.

7.3.4.3 Estimation of glutathione peroxidase activity (GPx), (E.C.1.11.1.9): Assay of glutathione peroxidase was carried out by continuous monitoring of NADPH oxidation in a recycling assay as described by Wendel (1981). Total GPx was measured by using cumene hydroperoxide as a substrate.

Reagents:
1. 0.25 M phosphate buffer, pH 7.0 containing 2.5 mM disodium ethylene diamine tetra acetic acid and 2.5 mM sodium azide.
2. Glutathione reductase: 0.3 U/mL.
3. 10 mM reduced glutathione (GSH).
4. 2.5 mM NADPH in 0.1% sodium carbonate.
5. 12.5 mM cumene hydroperoxide.

Procedure: The reaction mixture contained 0.1 mL of phosphate buffer, 0.1 mL glutathione reductase, 0.1 mL reduced glutathione and 0.1 mL of NADPH. To this 0.5 mL of homogenate was added and incubated at 37°C for 10 min. The reaction was started by the addition of 100 μL of cumene hydroperoxide. The linear decrease in absorption was recorded at 340 nm. The spontaneous reaction was assayed without enzyme and was subtracted from the samples. Activity of GPx was expressed as μmoles of GSH oxidized/min/ mg protein.

7.3.4.4 Estimation of glutathione reductase activity, (E.C.1.6.4.2): Glutathione reductase was assayed by the method of Staal et al., (1969).

Reagents:
1. 0.3 M sodium phosphate buffer (pH 6.8).
2. 250 mM EDTA
3. 12.5 mM glutathione oxidized (GSSG).
4. 3 mM Nicotinamide adenine dinucleotide phosphate reduced (NADPH).
Procedure: The reaction mixture containing 1 mL of phosphate buffer, 0.5 mL EDTA, 0.5 mL of glutathione oxidized and 0.2 mL of NADPH was made up to 3 mL with distilled water. After the addition of 0.1 mL of tissue homogenate, the change in O.D at 340 nm was monitored every 30 sec for 2 min. The enzyme activity is expressed as μmoles of GSH utilized/min/mg protein.

7.3.4.5 Estimation of glutathione-S-transferase (GST) activity, (E.C.2.5.1.18): Glutathione-S-transferase activity was determined by measuring the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitro benzene (CDNB) as a substrate by the method of Habig et al., (1974).

This enzyme catalyzes the reaction of CDNB with the sulfhydryl group of GSH to form CDNB-GSH conjugate that absorbs light at 340 nm.

Reagents:
1. 0.5 mM phosphate buffer, pH 6.5
2. 30 mM CDNB.
3. 30 mM reduced glutathione (GSH).

Procedure: The reaction mixture contained 2.7 mL phosphate buffer, 0.1 mL CDNB and 0.1 mL of the tissue homogenate which was incubated at 37°C for 10 min. The reaction was initiated by adding 0.1 mL of glutathione. The reaction was monitored spectrophotometrically for increase in absorbance at 340 nm. Measuring and subtracting the rate in the absence of enzyme made correction for the spontaneous reaction. The enzyme activity is expressed as units/min/mg protein.

7.3.5 Non-enzymic antioxidants

7.3.5.1 Estimation of reduced glutathione: Reduced glutathione was determined by the method of Moron et al., (1979).
Reagents:

1. Phosphate buffer, 0.2 M (pH 8.0).
2. Trichloroacetic acid, 10%.
3. 5,5’-dithiobis-2-nitro benzoic acid (DTNB), 0.6 mM in 1% sodium citrate.
4. Standard glutathione: 10 mg of reduced glutathione was dissolved in 100 mL of distilled water.

Procedure: An aliquot of 1 mL of the homogenate was precipitated with 10% TCA. The precipitate was removed by centrifugation. To an aliquot of the supernatant was added 2 mL of phosphate buffer, 0.5 mL of DTNB reagent and the final volume was made up to 3 mL with distilled water. Standard was also treated in a similar manner. The colour developed was read at 412 nm. The amount of reduced glutathione was expressed as µg/mg protein.

7.3.5.2 Estimation of ascorbic acid/Vitamin C: Vitamin C was estimated by the method of Omaye et al., (1979). Ascorbic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2, 4-dinitrophenyl hydrazine; this undergoes further rearrangement to form a product with an absorption maximum at 520 nm.

Reagents:

1. Trichloroacetic acid, 5%.
2. 2, 4-Dinitrophenyl hydrazine-Thiourea-Copper sulphate (DTC) reagent: 0.4 g thiourea, 0.05g copper sulphate and 3 g of 2, 4-dinitrophenyl hydrazine were dissolved in 100 mL of 9 N sulphuric acid.
3. Sulphuric acid, 65% (v/v).
4. Standard ascorbic acid: 50 mg of ascorbic acid was dissolved in 100 mL of 5% trichloroacetic acid.

Procedure: To 0.5 mL tissue homogenate, 0.5 mL of distilled water and 1 mL of 5% trichloroacetic acid were added, mixed thoroughly and centrifuged for 20 min. To 1 mL of the supernatant, 0.2 mL of DTC reagent was added and incubated at 37°C for 3 hr. Then 1.5 mL for 65% sulphuric acid was added, mixed well and the solutions were
allowed to stand at room temperature for another 30 min. The colour developed was read at 520 nm. Graded amount of standards was also treated similarly. The level of ascorbic acid was expressed as µg/mg protein.

7.4. RESULTS AND DISCUSSION

7.4.1. In vitro antioxidant activities of mango wine

7.4.1.1. DPPH radical scavenging activity: The free-radical scavenging potentials of the mango wine at different concentrations were analyzed by the DPPH method, and the results are shown in Figure 7.4. At all the concentrations tested from 20 to 100 µM, the wine from Alphonso showed the highest activity when compared to the wine from other cultivars where as Raspuri showed the lowest activity. Wines from Alphonso (42), Sindhura (47), Banginapalli (30), Totapuri (35), Neelam (38), Rumani (32) and Raspuri (37%) exhibited low radical scavenging activities at 20 µM total carotenoid concentrations whereas the standard antioxidant exhibited 50% activity however wines from Alphonso (91), Sindhura (90), Banginapalli (88), Totapuri (88), Neelam (87), Rumani (85) and Raspuri (81%) exhibited high radical scavenging activities at 100 µM total carotenoid, whereas the standard antioxidant showed 97% at same concentration. Radovanovic et al. (2009) reported that the high phenolic content in the Serbian red wines contributes to their increased antioxidant activity and the wines showed antioxidant behaviour in the range from 95.6 to 70%. However, De Beer et al. (2003) reported that the potency of red wine total phenols was twice that of white wines implying that the red wine total phenols are more effective free radical scavengers than those of white wine. The activity of the dealcoholised wine is attributed to its hydrogen-donating ability (Shimada et al. 1992). The antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not propagate further oxidation of the lipid (Sherwin 1978).
7.4.1.2. Inhibitory effect on LDL oxidation: Oxidative modification of LDL is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases (Steinberg et al. 1989), and the dietary antioxidants that protect LDL from oxidation may, therefore, reduce atherogenesis (Pin-Der 1998). The antioxidant activity of mango wine against rat LDL oxidation is shown in Figure 7.5. The polyunsaturated fatty acids of LDL were oxidized, and the malondialdehyde formed has been estimated by using the TBA method. The average induction time for copper mediated LDL oxidation was around 20 min without the addition of mango wine. The lag phase, which represents the resistance of LDL to undergo lipid peroxidation, was prolonged in the presence of mango wine during incubation at different time intervals. The mango wine protected LDL from oxidation as measured by the prolongation of the induction time of the formation of conjugated dienes.
The mango wine exhibited 19.3, 35.3 and 53.3% protection (average value) at the end of 2, 4 and 6h incubation at 20 μM concentration, 24.6, 41.7, and 58.4% protection at the end of 2, 4 and 6h incubation at 60 μM concentration, however, it was 31.7, 48.3 and 66.4% protection at the end of 2, 4, and 6h at 100 μM concentration. Fuhrman et al. (1995) reported in an in vivo study that the daily consumption of red wine (400 mL) not the white wine for 2 weeks reduced the susceptibility of plasma and LDL to lipid peroxidation. Amongst the wine tested, wine from the cv. Alphonso exhibited highest protection at all the concentrations, however, wines from Raspuri and Rumani exhibited lowest protective action, the variation in activity could be attributed to the phytochemicals present. Rifici et al. (1999) reported that red wine inhibits the cell mediated oxidation of lipoproteins in vitro than white wine, this effect of the red wine is due to its antioxidant components include flavonoids, catechins, quercetin and resveratrol but not due to its ethanol content. The result from this study indicates a dose-dependent inhibition effect of mango wine against LDL oxidation.
7.4.2. **Biochemical assay of glucose**: The serum glucose levels remained unaltered and there were no significant differences observed between groups. The serum glucose concentrations (mg/dl) were PFC (92.1±8.0), EtOH (103.4±6.4), MW (93.5±8.9), DMW (102.3±9.1) and RW (87.1±4.8), respectively, among different groups (Table 7.1). Villegas et al. (2004) demonstrated that individuals subjected to alcohol restriction have an increase in the blood glucose rate which is not observed for individuals with occasional, moderate and high consumption, who remain with unaltered glucose levels. Considering this, the beverages used in this study viz., EtOH or MW or DMW or RW does not change the plasma glucose levels in normal rats.

7.4.3. **Biochemical assay of protein**: The serum total protein (g/dl) concentrations were not altered significantly among the various groups studied. The serum levels of total proteins were as PFC (7.81±0.35), EtOH (8.75±1.11), MW (7.89±1.05), DMW (7.73±0.82) and RW (7.80±0.26), respectively did not change significantly (P<0.05). However, there was a slight increase in the serum protein concentrations in EtOH treated group when compared to other groups (Table 7.1.). This increase in the serum protein levels in EtOH treated group could be a mild sign of impaired renal function. The change in total protein levels of animals is common in certain situations. A high level of total protein may be due to the presence of a paraprotein (abnormal plasma protein) and dehydration, whereas reduced protein levels occur in hepatic disease, nephrotic syndrome, and malnutrition (Mani et al., 2012). Serum albumin constitutes approximately 60% of the plasma proteins. The increased serum levels of total protein could be due to increased serum levels of albumin.

7.4.4. **Effect of mango wine, ethanol and red wines on the serum levels of bilirubin, urea, uric acid and creatinine**: Bilirubin is a breakdown product of hemoglobin. It is transported from the spleen to the liver and excreted into bile. Serum bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides various normal functions, liver excretes the breakdown product of hemoglobin i.e. bilirubin into bile (Plaa and Hewitt 1982). The present study shows significant variations in the bilirubin concentration among the groups studied (Table 7.1). The serum bilirubin (mg/dl) concentration in the PFC group is 1.27±0.24 and it was
significantly increased to 3.62±0.14 in EtOH treated group. However, there were no significant differences observed among the MW (0.98±0.26), DMW (0.80±0.06) and RW (1.09±0.09) groups. This increase in bilirubin in EtOH treated group indicates the abnormal liver function which may be the result of higher synthetic function of the liver. Chander et al., (1987) reported a similar results in which there was a 2-fold increase in bilirubin level after administration of EtOH for 60 days. However contrary results were also reported by Adaramoye and Oloyede (2012) for the bilirubin concentration in STZ induced diabetic rats who reported a decrease in bilirubin concentration in EtOH treated group. Serum bilirubin is also one of the most sensitive markers employed in the diagnosis of hepatic diseases. Hyperbilirubinemia results from the increase of bilirubin concentrations in serum and its causes includes increased hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, and drugs (Kapitulnik et al., 1975). It provides useful information on how well the liver is functioning (Harper 1961). The above mentioned results consider as a good indicator for proving the safe of MW, DMW and RW.

**Serum urea, uric acid and creatinine:** The serum urea, uric acid and creatinine were non-protein nitrogen compounds and are the end products of protein metabolism and must be removed continually to ensure continued protein metabolism in the cells. Urea is the major end-product of nitrogen catabolism in human. The significant increase in serum urea, uric acid and creatinine levels in general suggests renal malfunction. Urea is synthesized in the liver, released in to the blood and is cleared (excreted) by the kidneys. The changes in plasma urea level are due to alteration of renal function (Baron, 1987).

Previous reports showed that some herbal preparations used for long period are associated with kidney injury and hence, serum urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Moshi et al., 2001). In the present study, the urea concentrations (mg/dl) in PFC group animals were 35.6±7.98, however, there was a significant (P<0.05) increase in the group treated with EtOH (46.07±10.03).
Table 7.1. Effect of mango wine, ethanol and red wine on non-protein nitrogenous substances in serum of control and experimental rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>92.1±8.0a</td>
<td>103.4±6.4b</td>
<td>93.5±8.9a</td>
<td>102.3±9.1b</td>
<td>87.1±4.8a</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>7.81±0.35a</td>
<td>8.75±1.11a</td>
<td>7.89±1.05a</td>
<td>7.73±0.82a</td>
<td>7.80±0.26a</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.27±0.24c</td>
<td>3.62±0.14d</td>
<td>0.98±0.26b</td>
<td>0.80±0.06a</td>
<td>1.09±0.09b</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>35.6±7.98a</td>
<td>46.07±10.03c</td>
<td>42.5±5.53b</td>
<td>40.4±4.71b</td>
<td>43.0±6.39b</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.49±0.21ab</td>
<td>1.86±0.91b</td>
<td>1.12±0.13a</td>
<td>1.38±0.28a</td>
<td>1.33±0.23a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.74±0.08ab</td>
<td>1.09±0.08c</td>
<td>0.80±0.03b</td>
<td>0.73±0.07ab</td>
<td>0.76±0.06ab</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D from six animals in each group. Values not sharing a common superscript letter in column differ significantly at p<0.05 (DMRT).

There were no significant differences among the animals treated with MW (42.5±5.53), DMW (40.4±4.71) and RW (43.0±6.39); however when compared to the control group animals these values were slightly high and less than that of the values reported for EtOH group animals (Table 7.1). Elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea, as a result of increased synthesis of arginase enzyme involved in urea production (Hooper et al., 1998).

Uric acid is the main end-product of nucleic acid and purine catabolism in human liver. Hyperuricemia or elevated serum uric acid levels results from high production (metabolic) or decreased excretion (renal) (Baron 1987). Table 7.1 illustrate the changes in the levels of serum uric acid for control rats and those rats administrated with wine. The uric acid levels (mg/dl) in EtOH group rats was significantly increased (1.86±0.91) when compared to the other groups viz., PFC (1.49±0.21), MW (1.12±0.13), DMW (1.38±0.28) and RW (1.33±0.23). Yoshida et al., (2001) reported that the serum uric acid levels increased with the amount of EtOH consumed on the habitual base and the acute phase base. Serum uric acid is known to be increased by EtOH via ethanol-induced activation of adenine nucleotide turnover, which is triggered by the acetate formed from EtOH. Production of uric acid via adenosine nucleotide turnover may occur in every tissue in the body, and uric acid may be increased
throughout the body, because the large amount of acetate formed from EtOH in the liver is probably released and utilized at other tissues (Puig and Fox, 1984). Moderate EtOH consumption seems to have the overall effect of reducing DNA damage, as shown by the decrease in serum uric acid and urinary 8-OHdG levels as observed by Yoshida et al., (2001).

Creatinine, a by-product from muscle mass, will affect its concentration in blood. Table 7.1 depicts the effect of different wine on serum creatinine levels in rats. The serum creatinine (mg/dl) levels were in PFC group animals (0.74±0.08), EtOH (1.09±0.08), MW (0.80±0.03), DMW (0.73±0.07) and RW (0.76±0.06) respectively. The above data shows that Serum creatinine levels were significantly (P<0.05) increased in rats treated with EtOH when compared with others. Treatment of rats with MW or RW prevented the ethanol-induced increase in serum creatinine and urea. The elevated serum creatinine levels in EtOH treated group could be due to the nephrotoxicity resulted because of ethanol treatment. Kidney is the second organ most frequently affected by many compounds and is vulnerable to damage because of larger perfusion and the increased concentration of excreted compounds that occur in renal tubular cells (Mohamed et al., 2003).

Blood Urea Nitrogen (BUN) only begins to rise after a marked renal parenchyma injury (Lin et al. 2007). There is considerable evidence that increased oxidative stress may participate in the pathogenesis of nephropathy. Ethanol caused severe damage to the tissues of liver and kidney and in the mean time caused an increase in the levels of creatinine, uric acid and urea. Based on the biochemical analysis of renal and hepatobiliary functions, such as the levels of urea, creatinine and alkaline phosphate value, Nzi et al. (2007) found that fruit juices and fruit products are generally tolerated by rats. Hence the protective action of MW, DMW and RW were evidenced in the serum as the elevated levels of both urea and creatinine were markedly lowered below those elicited by the nephrotoxicant.
7.4.5. Effect of mango wine, ethanol and red wine on serum lipid profile: The liver is a metabolically versatile organ responsible for the regulation of internal chemical environment and is particularly important in the synthesis and regulation of circulating lipids, lipoproteins, triglycerides, cholesterol, cholesterol esters and in the degradation of cholesterol and steroids. The metabolic effects of alcohol on the liver and lipid metabolism are known for many years and have been extensively studied from more than four decades. In the present study there was a significant (P<0.05) induction in triglycerides concentrations (mg/dl) in EtOH treated animals (55.22±4.10) when compared with PFC (47.95±5.25), MW (44.37±7.57), DMW (43.14±9.67), and RW (42.8±6.63) groups (Table 7.2.). This triglyceride increase in the serum of EtOH treated group could be as result of EtOH alone. However, moderate alcohol consumption is associated with a lower risk of cardiovascular disorders, and the pattern and amount of alcohol are of more importance than the type of alcoholic beverage. One of the underlying mechanisms for this beneficial effect is its influence on lipids especially the increase in plasma HDL-cholesterol (Brinton, 2010). In case of moderate drinking, 1-3 glasses a day for men and 1-2 glasses for women, hardly any effect was seen on triglycerides. However, excessive alcohol intake may cause hypertriglyceridemia not only postprandially, but also in the fasting state. This is mainly due to an increase in the synthesis of large VLDL particles in the liver. When alcohol consumption is accompanied by a meal containing fat, especially saturated fat, it has a significant additive effect on the postprandial triglyceride peak. This peak is for the most part the result of retardation of chylomicron breakdown and to some extent of that of VLDL remnants. Most likely, this should be attributed to an inhibition of lipoprotein lipase activity by alcohol. In case of moderate and regular alcohol intake, adaptation restores LPL activity. In some cases, alcohol may also be responsible for extremely high levels of triglycerides with an increased risk of pancreatitis.
Table 7.2. Effect of mango wine, ethanol and red wine on lipid profile in serum of control and experimental rats

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>105.40±8.21a</td>
<td>101.83±10.15b</td>
<td>94.87±7.60c</td>
<td>97.53±6.87ab</td>
<td>90.23±11.04</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>36.68±3.27a</td>
<td>42.91±7.53bc</td>
<td>41.18±2.63ab</td>
<td>47.69±4.12c</td>
<td>42.72±2.31bc</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>48.12±5.63a</td>
<td>42.83±2.82c</td>
<td>35.62±2.49b</td>
<td>34.54±5.62a</td>
<td>32.32±5.64b</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>47.95±5.25ab</td>
<td>55.22±4.10b</td>
<td>44.37±7.57a</td>
<td>43.14±9.67a</td>
<td>42.8±6.63a</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>19.58±3.04b</td>
<td>14.63±1.24b</td>
<td>15.29±3.24b</td>
<td>14.63±1.92b</td>
<td>14.17±2.31b</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D from six animals in each group. Values not sharing a common superscript letter differ in column significantly at p<0.05 (DMRT).

Fatty liver is an important feature of alcohol abuse and alcohol decreased fatty acids oxidation levels in the liver and that resulted in hepatic triglycerides accumulation (Lamb et al., 1994). Hypertriglyceridemia may also result from genetically determined disturbances in lipid metabolism but may also be secondary to conditions such as obesity, diabetes mellitus, hypothyroidism, the nephrotic syndrome, and the use of certain medication. Hyperlipidemia and hypercholesterolemia are reported as the major risk factors in lifestyle related diseases such as atherosclerosis and related cardiovascular complications. Hypertriglyceridemia in combination with abnormally low concentrations of HDL is one the most common atherogenic profile of lipid metabolism of high prevalence seen in Indian population (Enas and Mehta, 1995). It is well documented that a low level of HDL-C is indicative of high risk for cardiovascular disease; an increase in its level could potentially contribute to antiatherogenicity (Assmann and Nofer, 2003).

Alcohol consumption in moderate amount is cardio-protective and its beneficial effect is largely mediated through its impact on cholesterol. Da Luz et al. (1999) demonstrated that red wine as well as non-alcoholic wine products reduced the formation of atherosclerotic plaques significantly in hypercholesterolemic rabbits,
despite high blood lipids. In this experience the mechanisms of action of the wine were not clarified. Arteriographic and necropsy studies in humans have also demonstrated anti-atherogenic effect of alcohol. In the present study, there was a significant (P<0.05) increase in HDL levels in the ethanol and wine treated animals when compared to the control group. The serum HDL-cholesterol (mg/dl) levels were in PFC (36.68±3.27), EtOH (42.91±7.53), MW (41.18±2.63), DMW (47.69±4.12) and RW (42.72±2.31), respectively. Of all the treatments, significantly higher value was reported in DMW group. However, there was a significant (P<0.05) reduction in the LDL content. The LDL-cholesterol (mg/dl) levels were in PFC (48.12±5.63), EtOH (42.83±2.82), MW (35.62±2.49), DMW (34.54±5.62) and RW (32.32±5.64), respectively. However there was maximum reduction in the animals treated with RW. The serum total cholesterol (mg/dl) levels were in PFC (105.40±8.21), EtOH treated (101.83±10.15), MW (94.87±7.60), DMW (97.53±6.87) and RW (90.23±11.04), respectively. Similar to LDL, the serum total cholesterol concentration was reduced significantly in animals treated with ethanol but maximum reduction was observed in animals treated with wine (Table 7.2.). Similar to this study, Wakabayashi and Groschner, (2009) have found that ethanol intake reduces total cholesterol and non-HDL-C. Jin-ying et al. (2007) reported that high-fat diets significantly increased plasma total cholesterol and triglycerides in hamsters, which implies that a high-fat and high-cholesterol diet increases the risk of hyperlipidemia.

Red wine are capable of increasing serum HDL, which prevents cholesterol accumulation in peripheral cells and is protective against arteriosclerosis by transporting cholesterol from the arteries to the liver for its subsequent metabolism and excretion. Alcohol consumption increases the levels of HDL and may explain, at least partially, wine’s protective effect. However it was reported by Fuhrman et al. (1995) that in which healthy men received 400 mL/day of wine during 2 weeks indicated an increase of HDL with intake of red wine, but not with white wine. Further, Perret et al. (2002) observed dose-dependent increases in HDL and in Apo A-1 levels. Based on the ECTIM study (Enquete Cas-Temoins de l’Infarctus du Myocarde), which involved 561 men with myocardial infarction and 643 healthy men from France and Northern Ireland,
Marques-Vidal et al., (1995) reported a significant increase in HDL cholesterol, from 0.47 to 0.59 g/L, in men who consumed 2.3 ounces/day of alcohol, mostly in the form of red wine, compared with nondrinkers. Apolipoproteins A-I and A-II also increased significantly. But, Perret et al., (2002) observed an increase in HDL levels in people drinking 1.7 ounces of wine per day. Isolated HDL displayed an increase of 27% in all cholesteryl ester molecule species. This effect was associated with enrichment of the HDL particles in polyunsaturated phospholipids, in those containing arachidonic acids (+30%) and eicosapentaenoic acids (+90%), and especially in those containing C 20:5 (Omega 3), which is thought could be beneficial by itself against CAD.

7.4.6. Effect of mango wine, ethanol and red wine on the activities of serum marker enzymes

7.4.6.1. Serum ALT, AST, AP activities: The most commonly used serum biochemical parameters to detect liver damage are ALT and AST as hepatocellular markers, and alkaline phosphatase (ALP) and bilirubin as hepatobiliary markers (Tennant, 1999). AST and ALT are commonly used as laboratory markers for chronic excessive alcohol consumption, although they are more directly related to liver status and these values may give more specific information on the alcoholic aetiology of liver disease when interpreted together (Niemela, 2002). The ratio of AST to ALT of over two is suggestive of alcoholic aetiology, while the ratio for patients with non-alcoholic liver disease is normally below one (Rosman and Lieber, 1994). It was also known that serum AST, ALT, GGT and LDH levels were susceptible to hepatotoxin and serve as markers of liver damage, which promotes the release of such aminotransferases from hepatocytes into the bloodstream (Dogan and Celik, 2011). Hence, the assessment of the levels of AST, ALT and ALP also provides a good and simple tool to measure the protective activity of the target drug against the hepatic damage of the target compounds (Hewawasam et al. 2004). Low levels of AST is normally found in the blood, however, when the liver or heart is damaged, additional AST is released into the bloodstream. It rises within 6 to 10 h and remains high for 4 days.
Table 7.3. Effect of mango wine, ethanol and red wine on liver markers in serum of control and experimental rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>25.14±0.24a</td>
<td>79.02±1.52d</td>
<td>38.1±1.21bc</td>
<td>37.45±2.41b</td>
<td>38.46±3.1bc</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>46.23±3.1b</td>
<td>103.12±2.2d</td>
<td>55.5±1.41c</td>
<td>42.4±1.3a</td>
<td>55.9±2.3c</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>68.53±3.3c</td>
<td>295.65±9.5d</td>
<td>56.1±7.2b</td>
<td>44.8±3.5a</td>
<td>49.5±3.6a</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>23.16±1.2a</td>
<td>29.67±1.5c</td>
<td>24.42±1.8b</td>
<td>22.25±0.9a</td>
<td>24.51±2.3b</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>1030.84±20.1a</td>
<td>1858.54±24.3c</td>
<td>1224.66±27.2c</td>
<td>1058.54±15.3b</td>
<td>1137.8±16.7d</td>
</tr>
<tr>
<td>AMY (IU/L)</td>
<td>990.44±10.5b</td>
<td>1146.55±21.3b</td>
<td>1158.51±15.6b</td>
<td>1019.73±14.6a</td>
<td>1154.22±14.9b</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D from six animals in each group. Values not sharing a common superscript letter differ in column significantly at p<0.05 (DMRT).

The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver, since these enzymes are cytoplasmically located and released into the blood after cell damage and the abnormal variation in these marker enzymes reflects the overall change in metabolism that occurs during pathological conditions (Baron 1987). Alkaline phosphatase is the marker enzyme for plasma and endoplasmic reticulum (Shahjahan et al. 2004). Hepatic cells participate in a variety of metabolic activities and contain a number of enzymes. In any stage of liver injury, the transport function of hepatocytes is disturbed, which can result in the leakage of plasma membrane, thereby causing an increase in SGPT and SGOT levels. In addition, a defective excretion of bile by the liver was reflected by an increase in the level of serum ALP. (Yanardag and Tunali, 2006).

Alcohol is one of the factors most frequently associated with increased liver enzyme and the association between alcohol intake and alcohol-induced liver disease is well known. It was documented that alcohol causes modifications in the fluidity of membranes, permeability of these membranes and their lipid composition (Hoek et al., 1988). Therefore, alcohol may exert its effect through alteration of synthesis in the endoplasmic reticulum, intracellular translocation and/or possibility of solubilization at the site of plasma membrane, hence increasing the level of serum enzymes especially membrane-bound enzymes, like ALP, and cytosolic enzymes, such as LDH and
transaminases (ALT and AST). It was shown in previous studies that chronic alcohol intoxication depresses hepatic enzyme activities, suggesting that elevated serum enzyme activities might be induced as a result of enhanced release of hepatic enzymes into blood stream due to liver cell injury (Singer and Kaplan, 1978). Elevated serum levels of ALP and LDH were also observed by Yokoyame et al. (1993) induced by ethanol. The Italian Dionysos study showed that alcohol was suspected to be the cause in 23% of all cases of liver disease, with a dose-dependent increase in the risk of developing liver disease (Bellentani et al., 1994). Therefore, the high AST and ALT levels in the serum of rats to which the ethanol or wines (red wine or fruit wine) were administered are indications of leakage into the bloodstream due to liver damage (Jacobs, 1996). In the present study, The AST (IU/L) level in PFC group animals was (25.14±0.24), EtOH (79.02±1.52), MW (38.1±1.21), DMW (37.45±2.41) and RW (38.46±3.1) indicating a significant increase in serum AST in animals treated with ethanol. However there was only a non-significant increase in wine treated animals. However the ALT (IU/L) level in PFC animals was (46.23±3.1), EtOH (103.12±2.2), MW (55.5±1.41), DMW (42.4±1.3) and RW (55.9±2.3). The ALP (IU/L) levels in PFC group animals was (68.53±3.3), EtOH (295.65±9.5), MW (56.1±7.2), DMW (44.8±3.5) and RW (49.5±3.6) indicating a significant increase in both serum ALT and ALP in animals treated with EtOH (Table 7.3.). Significantly increased levels of serum enzymes such as AST, ALT, ALP, and GGT in the present study have been observed in EtOH administered rats, which indicate the increased permeability, damage, and/or necrosis of hepatocytes (Pari and Karthikesan, 2007). The animals treated with MW or DMW did not shown significant alterations in the levels of AST, ALT, ALP, GGT, total protein, albumin and direct bilirubin content, indicating that wine did not cause damage in the hepatocytes and the biliary system.

7.4.6.2. $\gamma$-glutamyl transferase (GGT): Serum $\gamma$-glutamyl transferase (GGT) is an enzyme produced predominately in the liver. It is involved in glutathione metabolism and in the renal reabsorption of amino acids. An increased serum level of GGT serve as an indicator of hepatobiliary disease and was also considered as a marker of oxidative stress (Lee et al., 2004) because it catalyzes the first step in the degradation of
extracellular glutathione. It is a sensitive marker of alcohol ingestion, especially when chronic alcoholic liver disease results. Serum levels of GGT have been found to be elevated in about 75% of individuals who are alcohol-dependent, with a range in sensitivity of 60-90%. The sensitivity is greatest when alcoholics and chronic heavy drinkers are compared to teetotalers and infrequent social drinkers and is primarily an indicator of chronic consumption of large amounts of alcohol and is not increased by binge drinking in non-alcohol abusers, unless there is concomitant liver disease. The GGT (IU/L) levels in the present study were in PFC (23.16±1.2), EtOH (29.67±1.5), MW (24.42±1.8), DMW (22.25±0.9) and RW (24.51±2.3), respectively (Table 7.3.). A significant increase in GGT levels was noted in ethanol group, which might be induced by ethanol alone in the present study. The GGT levels in DMW group was slightly lower than that of the control which shows that it was not induced or reverted in absence of alcohol. However there was slight increase in GGT levels in MW and RW groups when compared to PFC but the values were not higher than that of ethanol group. The half-life of GGT is between 14 and 26 days and its level usually returns to normal in 4-5 weeks after drinking ceases. Elevations are not specific for alcohol damage since increases also occur in liver disease and with certain drugs (Goldberg and Kapur, 2004). In addition serum GGT concentration may be elevated by diabetes, obesity and certain drugs and diseases, including biliary tract disease, severe heart and kidney diseases, trauma and hyperthyroidism (Allen et al. 2000). It was proved that people with high serum GGT have higher mortality, partly because of the association between GGT and other risk factors and partly because GGT is an independent predictor of risk. In addition, the relationship between alcohol intake and serum GGT values differs with gender, and also probably varies with age, obesity, smoking, and other individual characteristics. The function of GGT on the cells is by protecting cells against free radical injury, particularly in regions of inflammation; and second, that GGT participates in events that modify receptor-ligand interactions at the cell membrane (Whitfield, 2001)
7.4.6.3. Lactate dehydrogenase (LDH): Lactate dehydrogenase (LDH) is a key enzyme of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalysed by LDH interlinks anaerobic and aerobic oxidation of glucose (Robert et al., 2000). LDH is found in the cells of most tissues including the heart and liver. Elevation of serum LDH is observed due to in vivo effects of toxic agents like clofibrate, dicumarol, ethanol, carbon tetrachloride etc. Owing to its widespread distribution in the tissues, elevation of the total LDH in the serum is generally of little value in diagnosis. High values commonly occur after myocardial infarction, in megaloblastic anemia, progressive muscular dystrophies and in neoplastic diseases especially widely disseminated forms. In the present study, The LDH activities (IU/L) were significantly increased in EtOH treated group (1858.54±24.3) when compared to other groups and PFC animals (1030.84±20.1). The LDH activity of MW, DMW and RW treated were 1224.66±27.2, 1058.54±15.3 and 1137.8±16.7, respectively (Table 7.3). The results are in agreement with Bharrhan et al. (2011) who reported a significant increase in LDH activity in the serum and liver homogenates of EtOH fed rats. The LDH activity was found to be directly proportional to the concurrent reduction of NAD+ which is used for the oxidation of alcohol to acetaldehyde (Nine et al., 1995). NADH+H+ generated may be utilised by pyruvate which results in the formation of excess lactate and thereby increases the LDH activity.

7.4.6.4. Amylase: Chronic excessive consumption of alcoholic beverages is clearly associated with acute and chronic pancreatitis. While the role of alcohol (ethanol) in the development of pancreatitis has been intensively investigated over the past three decades and found that ethanol administration resulted in a transient increase of pancreatic amylase output and plasma cholecystokinin (CCK) levels. Pure ethanol at low concentrations (<4% v/v) is a mild stimulant of gastric acid output with a response equal to about 23% of the pentagastrin-stimulated gastric acid output however higher concentrations of ethanol (5-40% v/v) have either no effect or a mildly inhibitory one (Singer et al., 1987). In the present study, the serum amylase (IU/L) levels in PFC (990.44±10.5), EtOH (1146.55±21.3), MW (1158.51±15.6), DMW (1019.73±14.6) and
RW (1154.22±14.9) groups, respectively. There was slight increase in amylase levels in both mango and red wine groups when compared to control however there was no significant increase in DMW (Table 7.3), which could be due to the reason that the beverage was devoid of alcohol. In ethanol fed group, the amylase levels were very slightly lower than that of mango and red wine groups which might be due to the fact that alcoholic beverages with low ethanol content (e.g., beer and wine) are powerful stimulants of gastric acid output and gastrin release. Distilled alcoholic beverages with higher ethanol content (e.g., whisky and cognac) do not stimulate gastric acid output or release of gastrin (Singer et al., 1987). However Lankisch et al., (1999) showed that patients with a large amylase increase showed minimal or moderate pancreatitis, those with a smaller amylase increase were found to have severe pancreatitis at surgery and hence their study suggests that the severity of the disease must be evaluated independently of the enzyme level elevation at the time of initial diagnosis, as the complication rate, indication for surgical treatment, and death rate are not related to the initial enzyme increase.

7.4.7. Effect of mango wine, ethanol and red wine on the activities of antioxidant enzymes in liver and kidney tissues: Ethanol, a commonly abused substance by society, has been shown to produce oxidative stress in vital tissues of the body. It reacts relatively non-specifically thereby giving it the ability to influence a wide range of cellular targets rather than a singular site and evidence reveals that the metabolism of ethanol gives rise to the generation of excess amounts of free radicals (Bondy et al., 1992; Guerrie et al., 1994). Acute ingestion of ethanol has been related to the formation of ROS (Bondy and Orozco, 1994). Ethanol is extensively metabolized to acetaldehyde in the liver by alcohol dehydrogenase (ADH), and is further oxidized to acetate with the generation of superoxide (Nordmann et al., 1987). Ethanol is also metabolized specifically by hepatic microsomal Cytochrome P450 II E1 resulting in the generation of alpha-hydroxyethyl radicals (Reinke et al., 1994). Hence, the liver is highly susceptible to the oxidative events associated with the toxicity of ethanol.

The kidney, which is the site for elimination of reactive metabolites may also be effected by ethanol induced alpha-hydroxyethyl radical oxidant species. Chronic
ethanol studies have shown increases in ethanol oxidation as well as lipid peroxidation in the kidney (Orellana et al., 1998), however the effect of acute ethanol on renal antioxidant defense is sparse.

As liver and kidney were the two important organs which play important role in physiological aspects, any damage in these tissues can lead to alterations in metabolic activities including cellular antioxidant status. The biological effects of ROS are controlled in vivo by a wide spectrum of enzymatic and non-enzymatic defence mechanisms such as SOD which catalyzes the dismutation of superoxide anions into hydrogen peroxide, CAT which detoxifies H$_2$O$_2$ and GPx which converts hydroperoxides into nontoxic alcohols. Nonenzymatic antioxidants such as vitamins E and C, and poly phenolic compounds represent some of these natural antioxidants that could act to overcome the oxidative stress. Hence the research was focused on investigating the effects of alcohol and protective role of wines on the levels of antioxidant enzyme activities, GSH, Vitamin C, lipid peroxidation in the liver and kidney of rats.

7.4.7.1 Superoxide dismutase (SOD) and catalase (CAT): SOD and CAT are two important enzymatic antioxidants that act against toxic oxygen free radicals such as superoxide (O$^{2-}$) and hydroxyl ions (OH$^-$) in biological systems and contribute to the tissue oxidative/antioxidative balance. Catalase is present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. However, SOD was considered as the first line of defense against oxygen toxicity and the central regulators of reactive oxygen species (ROS) levels by catalyzing the decomposition of superoxide, the first but most abundant ROS, into hydrogen peroxide and water. Over-expression of SOD reduces oxidative damage and extends life span, while mutation in SOD reduces oxidative stress resistance and cell viability with decrease of SOD expression or activity (Landis and Tower, 2005). However, the decrease of catalase activity may cause the accumulation of the O$^{2-}$, H$_2$O$_2$ or their product of decomposition. Loss of catalase activity results in oxygen intolerance and triggers a number of deleterious reactions such as protein and DNA oxidation, and
cell death (Halliwell and Gutteridge, 1999). In the present study, there was a significant decrease in the activity of SOD (U/mg protein) in liver and kidney, respectively in EtOH treated group (6.6±2.3 and 8.62±0.8) when compared to PFC group (13.0±1.23 and 15.4±1.5) and also with other groups. However, it was observed that the activity of SOD increased significantly (P<0.05) in animals fed with MW (15.3±2.1 and 15.0±1.1), DMW (16.1±2.5 and 16.5±1.4) or RW (16.5±1.8 and 16.8±0.99) in both liver and kidney (Table 7.4.) when compared to the control group. This decrease in SOD activity in response to ethanol in the liver might be due to the fluxes in SOD activity in either direction may relate to the presence of excess ROS (Bondy, 1992). Therefore, the decrease in SOD activity after administration of ethanol indicates an oxidative stress response by the liver's defense system. A decrease in SOD activity generally reflects the inability of a tissue to scavenge excess superoxide anions leading to oxidative stress (Jenkins and Goldfarb, 1993). Similar to the SOD activities, there was a significant decrease in the CAT activity (U/mg protein) in liver and kidney respectively in EtOH treated group (58.6±4.5 and 32.45±2.1) when compared to PFC group (76±10.2 and 45.2±1.6) and also with other groups. However, it was observed that the CAT activity increased significantly (P<0.05) in animals fed with MW (80.4±6.4 and 51.8±6.7), DMW (84.3±8.1 and 55.7±5.5) and RW (86.4±7.6 and 56.3±3.4). In the present study, similar trend in the SOD and CAT activities of both the liver and kidney tissues was observed on exposure to ethanol and MW or RW. Those animals treated with wine (mango or red wine) showed good antioxidant activity by a free radical scavenging mechanism, which could have beneficial action against pathological alteration caused by ROS. Similar to this study, Scott et al. (2000) reported an decrease in SOD and CAT activities of the liver following ethanol ingestion, which might be due to inactivation of enzyme protein on account of alpha-hydroxy ethyl radical generation mainly in the liver where ethanol is extensively metabolized compared to lung and kidney. A decrease of both SOD and Cat activities in both liver and kidney of rats exposed to ethanol might lead to the accumulation of ROS and consequently to an increase in MDA concentration. In the present study, ethanol induced inhibition of SOD and Cat activity suggests an important role for these enzymes in the pathogenesis of the tissue injury. Further, Brzozowski et al. (1998) demonstrated that ethanol decreased the gene
expression and the activity of SOD in the gastric mucosa, suggesting that the suppression of key mucosal antioxidant enzyme, along with the elevation of lipid peroxidation, play an important role in the pathogenesis of gastric lesions.

7.4.7.2 Glutathione peroxidase (GPx), Glutathione-S-transferase (GST) and Glutathione reductase (GR): It is a well-known first line defense antioxidant enzyme of the cell against oxidative challenge, and it requires glutathione as a co-substrate. GPx is a selenium dependent enzyme that catalyzes the breakdown of $H_2O_2$ and organic hydroperoxides thus preventing intracellular damage caused by the free radicals. GPx also catalyzes the reduction of organic hydroperoxides or lipid peroxides to their respective alcohols in the presence of GSH. GPx coupled with glutathione reductase, catalyses the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) and simultaneously NADPH is oxidized to NADP+. It also plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging ROS. The elevated GPx activity in general may serve as a protective measure against further peroxidative damage in the tissue and however its decreased activity might be attributed to increased utilization for elimination of $H_2O_2$ and organic hydroperoxides. In the present study, there was a significant decrease in GPx activity (U/mg protein) in liver and kidney, respectively in EtOH treated group (0.68±0.01 and 0.48±0.04) when compared to PFC group (0.93±0.06 and 0.72±0.02). However, it was observed that the activity of GST increased significantly (P<0.05) in animals fed with MW (1.06±0.03 and 0.84±0.01), DMW (1.15±0.04 and 0.88±0.04) or RW (1.18±0.08 and 0.91±0.01) in both liver and kidney. The present study reveals that the activity of GPx was significantly decreased in ethanol treated rats in both the tissues. Ostrowska et al. (2004) have reported a decrease GPx activity in the ethanol treated rats and the decreased GPx activity may disturb the glutathione homeostasis in liver cells and that ultimately leads to the damage of hepatocytes. Decrease in GPx activity may be due to either free radical dependant inactivation of enzyme or depletion of its co-substrate, i.e., GSH and NADPH in the ethanol treatments (Chandra et al., 2000). Liver GSH content after alcohol administration was found to decrease, due to increased utilization by the hepatocytes. In such conditions, due to non-availability of GSH, a decrease in the
activity of GPx has been observed in the hepatocytes. Decreased hepatic GPx activity due to ethanol consumption indicate low profile in scavenging the hydrogen peroxide and lipid peroxides, which may be due to oxidative inactivation of the enzyme (Pigeolet et al., 1990). The reason for the reduced activity of GPx might lie in the decreased mitochondrial pool size of glutathione, because the entry of cytosolic glutathione into mitochondria is impaired (Kerem and Koren, 2003).

Glutathione-S-transferases (GST) are a multigene superfamily of dimeric, multifunctional, and soluble enzymes that play an essential role in protecting organisms from oxidative damage to DNA and lipids, localized in the cytosol, mitochondria and membrane of cells. They are phase II detoxification enzymes that, through the action of conjugation with glutathione, metabolize xenobiotics such as carcinogens and pollutants, and by-products of oxidative stress. GST plays an important role in the detoxification of toxic electrophiles by conjugating them with glutathione. G6PDH is involved in GSH synthesis by donating NADPH for GR to reduce oxidized glutathione (GSSG) to reduced glutathione (GSH). The decline in the activities of GR and GST may be due to the involvement of these enzymes in the detoxification and possibly repair mechanism in liver and kidney. Induction of these enzymes has been evaluated as a means for determining the potency of many antioxidant substances. In the present study, there was a significant decrease in GST activity (U/mg protein) in liver and kidney respectively in ethanol treated group (7.6±1.4 and 5.8±0.55) when compared to PFC group (14.6±1.1 and 11.6±0.92). However, it was observed that the activity of GST increased significantly (P<0.05) in animals fed with MW (15.4±1.2 and 12.2±1.1), DMW (15.8±2.2 and 12.9±2.4) and RW (16.4±1.8 and 13.2±1.4) in both liver and kidney. Similar decrease in GST levels was reported by Guven and Kaya, (2005) in Goose liver cells with damage induced by ethanol. Alin et al., (1985) reported that ethanol or its metabolic products might specifically target GST isoenzymes and the reduction in enzyme activity or expression might contribute to ethanol hepatotoxicity.

The decreased activities of GPx and GST in EtOH treated group could be directly explained by the low content of GSH found in these rats since GSH is a substrate and co-factor of GPx and GST. GSH, the most important antioxidant
metabolite, plays an important role in maintaining good levels of GPx activity. Flohe (1971) reported that the kinetics of GPx is in the first order with respect to GSH, hence the decreased levels of GSH in EtOH treated group may be one of the factors for decreased activity of GPx. The low activity of GPx causes accumulation of H2O2. GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress. The depletion in the activities of GST and GPx may result in the involvement of deleterious oxidative changes due to accumulation of toxic products. Thus, the decreased activities of these GSH related antioxidant enzymes may be responsible for elevated LPO observed in ethanol fed rats.

Glutathione reductase (GR) is one of the GSH-related enzymes which play detoxifying and antioxidant roles in metabolizing xenobiotics through the conjugation with glutathione or reduction of free radicals. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidised glutathione (GSSG) to reduced form (GSH) by the oxidation of NADPH to NADP+ and GPx worked together with GSH in disintegrating hydrogen peroxide or other organic hydroperoxides (Hatono et al., 1996). Deficiency of this enzyme will affect the redox status of GSH in biological system and could not protect tissues from oxidative damage.

In the present study, there was a significant decrease in the activity of GR (U/mg protein) in liver and kidney respectively in EtOH treated group (1.1±0.89 and 0.92±0.14) when compared to PFC group (2.4±0.1and 2.2±0.1) and also with other groups. However, it was observed that the activity of GR increased significantly (P<0.05) in animals fed with MW (2.6±0.21 and 2.7±0.18), DMW (2.9±0.22 and 2.8±0.19) or RW (3.2±0.18 and 3.3±0.15) in both liver and kidney, respectively (Tables 7.4 and 7.5). This significant reduction in the GR activity after ethanol ingestion was also observed by Scott et al., (2000) and this decrease in GR after ethanol is indicative of impaired reduction of GSSG to GSH due to the depletion of the reducing equivalent NADPH, which is a co-substrate required for GR activity.
Table 7.4. Effect of mango wine, ethanol and red wine on enzymic and non-enzymic antioxidants in liver tissue of control and experimental rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
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</thead>
<tbody>
<tr>
<td>Superoxide Dismutase (U/mg protein)</td>
<td>13.0±1.23b</td>
<td>6.6±2.3a</td>
<td>15.3±2.1f</td>
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<td>Catalase (U/mg protein)</td>
<td>76±10.2b</td>
<td>58.6±4.5a</td>
<td>80.4±6.4c</td>
<td>84.3±8.1c</td>
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<td>Glutathione peroxidase (U/mg protein)</td>
<td>0.93±0.05b</td>
<td>0.68±0.01a</td>
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<td>1.15±0.04d</td>
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<td>Glutathione reductase (U/mg protein)</td>
<td>2.4±0.10a</td>
<td>1.1±0.89a</td>
<td>2.6±0.21b</td>
<td>2.9±0.22c</td>
<td>3.2±0.18c</td>
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<td>Glutathione S-transferase (U/mg protein)</td>
<td>14.6±1.1b</td>
<td>7.6±1.4a</td>
<td>15.4±1.23c</td>
<td>15.8±2.2kc</td>
<td>16.4±1.8c</td>
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<td>Reduced Glutathione (μg/ g tissue)</td>
<td>540.5±30.3c</td>
<td>180.5±37.7a</td>
<td>520.8±26.4b</td>
<td>553.7±31.1c</td>
<td>566.8±22.4d</td>
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<td>Vit C (μ mole/ mg tissue)</td>
<td>2.5±0.01e</td>
<td>0.95±0.01a</td>
<td>2.1±0.03c</td>
<td>2.25±0.02d</td>
<td>2.15±0.03c</td>
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<tr>
<td>LPO (nmoles MDA/mg protein)</td>
<td>2.5±0.1a</td>
<td>6.2±0.2c</td>
<td>2.7±0.2b</td>
<td>2.6±0.2b</td>
<td>2.8±0.2b</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D from six animals in each group. Values not sharing a common superscript letter differ in column significantly at p<0.05 (DMRT).
Table 7.5. Effect of mango wine, ethanol and red wine on enzymic and non-enzymic antioxidants in kidney tissue of control and experimental rats.

<table>
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<tr>
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<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide Dismutase (U/mg protein)</td>
<td>15.4±1.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>16.8±0.99&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Glutathione reductase (U/mg protein)</td>
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<td>2.7±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3.3±0.15&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
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<td>5.8±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.2±1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.9±2.4&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Reduced Glutathione (µg/ g tissue)</td>
<td>475.6±31.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>156.4±12.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>480.46±34.5&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Vit C (µ mole/ mg tissue)</td>
<td>1.48±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.67±0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.32±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.44±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>LPO (nmoles MDA/mg protein)</td>
<td>2.36±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.18±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.64±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.74±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.68±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D from six animals in each group. Values not sharing a common superscript letter differ in column significantly at p<0.05 (DMRT).
In the present study, there were decreased activities of GPx and GR in liver and kidney result in the involvement of deleterious oxidative changes and also insufficient availability of reduced glutathione (GR) also number of deleterious effects due to the accumulation of toxic products.

7.4.7.3 Effect of mango wine, ethanol and red wine on the lipid peroxidation in liver and kidney tissues: Reactive oxygen species (ROS) are generated by many redox processes that normally occur in metabolism of aerobic cells. If not eliminated, ROS can attack important biological molecules, such as lipids, proteins, DNA, enzymes, and RNA. Thus, ROS are involved in a number of degenerative diseases such as cancer, cirrhosis, diabetes, and Alzheimer’s disease. ROS are generated by many redox processes that normally occur in metabolism of aerobic cells. However, ROS generated are scavenged by the antioxidant defense system. In tissues, the cellular antioxidant defense system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), and antioxidants such as glutathione (GSH) and vitamins A, C and E. Oxidative stress results in when the production of free radicals or ROS in the tissues exceed the ability of the antioxidant system to eliminate them. Lipid peroxidation explain a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival and membrane fluidity (Thampi et al., 1991). The increase in the levels of TBARS indicates an enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals (Comporti, 1985). Any compound – natural or synthetic – with antioxidant activity might totally or partly alleviate this damage (Sepici-Dincel et al., 2007). Carotenoids are also well known for their proantioxidant ability, in which they have shown membrane protection ability mainly by lipid peroxidation prevention and restoration of various antioxidant enzymes like superoxide dismutase, catalase, and peroxidases (Palozza and Krinsky, 1992). The formation of ROS was prevented by an antioxidant system that included non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and ROS-scavenging enzymes such as SOD, CAT and GPx (Lin et al., 2005).
Ethanol is able to induce the formation of free radicals and during its biotransformation, occurring with the involvement of the microsomal ethanol-oxidizing system (MEOS), xantine oxidase and aldehyde oxidase, ROS and hydroxyethyl radicals are generated. EtOH, apart from being a source of free radicals, can also disturb the antioxidant defence system (Montoliu et al., 1994). The elevation in lipid peroxidation in the liver and kidney of ethanol treated rats might be a consequence of increased formation of free radicals as well as the inhibition of SOD and CAT activities (Jurczuk et al., 2004). The results obtained indicate that during the ethanol treatment, the antioxidant defence system in the liver was insufficient to give complete protection and thus the processes of lipid peroxidation escalated. Despite more effective antioxidant defence system in liver, the ethanol induced disturbances were more seriously advanced in the liver. It might result from the fact that the liver is the main site of ethanol biotransformation and at the same time the main site of free radicals formation.

7.4.7.4 Effect of mango wine, ethanol and red wine on the non-antioxidants Vitamin C and GSH in liver and kidney: GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense process. It is the most abundant intracellular thiol based antioxidant present in millimolar concentration, and it serves as a significant first line of defense against the oxidative stress, and it plays an important role in maintaining the integrity of cells. GSH depletion increases the sensitivity of organ to oxidative and chemical injury. Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion (Ahmed and Zaki, 2009) its Perturbation in biological system has been reported to lead to serious consequences. Liver is the primary organ for the metabolism of ethanol where free radicals and ROS are generated. Results indicate that ethanol ingestion significantly decreased hepatic GSH content in a dose dependant manner. Endogenous GSH plays an important role in the scavenging ROS and in the detoxification of drugs and chemicals in the liver (Somani et al., 1996). It was reported by Scott et al. (2000) that liver is highly susceptible to oxidative damage caused by ROS and glutathione depletion when compared with lung. Further it was also showed that hepatic GSH depletion above 20% has been shown to impair the cell's defense
against ROS, and has been known to cause hepatic injury (Deleve and Kaplowitz, 1990). Thus, depletion of hepatic GSH in the present study in ethanol treated rats is indicative of ethanol induced hepatic injury. The observed decreases in GSH levels are consistent with past acute studies which showed depletion in vitro and in vivo after ethanol (Oh et al., 1997). Similar to the present study, Kim et al. (2003) have reported decreased hepatic GSH levels in ethanol treated 4, 12 and 50 weeks old female Sparague-Dawley rats. The depletion of GSH seems to be the prime factor that permits lipid peroxidation in the ethanol treated group. The protection of GSH is by forming the substrate for GPx activity that can react directly with various aldehydes produced from the peroxidation of membrane lipid in wine treated rats.

L-Ascorbic acid (AA), commonly known as vitamin C, is considered one of the organism’s most powerful antioxidant agents due to its capacity to donate two electrons from its double bond and is an excellent hydrophilic antioxidant in plasma because it disappears faster than other antioxidant when plasma is exposed to ROS. It is an essential vitamin because it participates in reactions such as the synthesis of molecules such as carnitine or thrysine acid, which are fundamental for bodily function. It also participates in iron absorption and presents immunological and anti-inflammatory actions such as the synthesis of neurotransmitters and hormones, playing an important role in collagen synthesis (Morales-Gonzalez, 2009). The functions of vitamin C apparently reflect its redox capacity. Thus, participates in some hydroxylation reactions in which it maintains optimal enzymatic activity by means of electron donation. Vitamin C also increases the absorption of non-heme iron and serves as an important mechanism for inactivating highly reactive radicals in tissue cells. Similarly, it delays the formation in the body of nitrosamines, which are possible carcinogens. The accumulated evidence links ascorbic acid with many elements of the immunitary system (Morales-Gonzalez, 2009). Vitamin C also plays an important role in detoxification of reactive intermediates produced by cytochrome P450, which detoxify xenobiotics (Prakasam et al., 2005). It regenerates membrane-bound α-tocopherol radical and removes the radical from the lipid to the aqueous phase. Vitamin C has been reported to
contribute up to 24% of the total peroxyl radical-trapping antioxidant activity (TRAP) (Atanasiu et al., 1998).

In the present study, there was a significant decrease in the activity of ascorbic acid (μ mole/ mg tissue) in liver and kidney, respectively in EtOH treated group (0.95±0.01 and 0.67±0.01) when compared to PFC group (2.5±0.01 and 1.48±0.01) and also with other groups MW (2.1±0.03 and 1.32±0.02), DMW (2.25±0.02 and 1.44±0.02) or RW (2.15±0.03 and 1.38±0.03) in both liver and kidney, respectively. Ascorbic acid concentration was found to be significantly decreased in the liver and kidney of ethanol treated rats. This decrease in ascorbic acid level in tissues (liver and kidney) may be due to increased utilization for scavenging the free radicals and decreased GSH level, which is required for recycling of vitamin C. Increased GSH levels upon treatment also helps in the recycling of vitamin C. The levels of ascorbic acid and GSH were decreased in cases with liver diseases, particularly in alcoholics (Bjorneboe et al., 1987).

**Histopathology:** The histological sections of the liver and kidney tissues were observed to evaluate any protective or harmful effects of MW, DMW and RW, comparing with that of EtOH. The photomicrographs are presented in Figures 7.6 and 7.7. The changes in the liver in rats induced by ethanol have been reported earlier (Pari and Karthikesan, 2007). The liver of rats treated with EtOH showed degeneration and congestion. However no significant alterations were observed in liver slides of MW or DMW or RW and the absence of morphological alterations further supports the protective role of nonalcoholic components of wine from ethanol-induced deleterious events. However, wine consumption can induce cirrhosis, most particularly when associated with deficient nutrient intake.

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Any structural changes in kidneys resulting from EtOH administration in rats can thus be attributed to altered metabolism during the treatment.
Fig. 7.6. Photomicrograph (×40) showing effect of administration of mango wine, dealcoholised mango wine, ethanol and red wine on architecture of liver of rats as compared to control.
Fig. 7.7. Photomicrograph (×40) showing effect of administration of mango wine, dealcoholised mango wine, ethanol and red wine on architecture of kidney of rats as compared to control.
Not much significant alterations were observed in liver slides of MW or DMW or RW but the kidney slides of rats treated with EtOH showed severe congestion.

In conclusion, MW and or DMW can be considered safe as it did not cause any lethality or adverse changes in the general behavior and also no detrimental effects caused by these beverages in biochemical and histopathological changes in rat model were observed. This shows the non toxic nature of the mango wine and or dealcoholised mango wine. Thus these can be safely administered for further in vivo studies to study their antioxidant activities in humans.

7.5 CONCLUSIONS

The present study shows noticeable improvements of several hepatic parameters following mango wine, dealcoholised mango wine or red wine consumption in contrast with the rats with similar ethanol intake. This striking difference may be related to the nonalcoholic components of wine and highlights the importance of considering the biological matrix other than alcohol while evaluating the effects of alcoholic beverage intake.