Discussion
5.1 Mortality and Sickness

In the present study, no mortality was observed at the dose of 4 mg/kg b.wt./day sodium arsenite (NaAsO$_2$) for 30 days in arsenic treated group. Sodium arsenite produced sickness in animals in terms of lethargy, loss of appetite, diarrhoea and hair loss.

Early clinical symptoms at acute arsenic intoxication may be weakness, with flusking skin, reddish rashes in the body and skin becomes cold and clammy in humans (Saha et al., 1999). Though diarrhoea is a major and early onset symptom in acute arsenic poisoning, in chronic toxicity diarrhoea occurs in recurrent bouts and may be associated with vomiting. Suspicion of arsenic ingestion should be aroused if other manifestations such as skin changes and a neuropathy are also present (Poklis and Saady, 1990). Balch (2008) revealed that hair loss is included in one of the symptoms of arsenic poisoning. Diffuse alopecia related to ingestion of toxic metals from the environment has been observed in 36 patients. Copper, arsenic, mercury and cadmium were involved and the intensity of disturbance of the hair cycle, heralded by presence of dystrophic hairs, was proportional to the amount of toxic material detected in blood and urine. Toxicity can be observed in vitro by measuring labeled cells on squash preparations after incorporation of tritiated uridine and thymidine in plucked hairs. The labeling indexes, as well as the ratio between cells entering the S phase and the viable cells were always lower in toxic alopecia than in normal hair (Pierard, 1979).

Dart (2003) and Tatum (2009) have reported that arsenic poisoning induces lethargy. In a case study, a 54-year-old woman reported hair loss after arsenic ingestion (EHP, 2007).

Pike-Paris (2004) also reported that Girls and boys ingested sodium arsenate shows pale and lethargic. In a survey done in Nepal, village people
drinking water contaminated with arsenic (around 300 ppb), were noticeably more lethargic as compared to those drinking filtered water (Bms world mission, 2009). ATSDR (2011) also described symptoms of arsenic poisoning such as weakness and lethargy.

Yadav et al. (2009) demonstrated that a significant decrease in locomotor activity, grip strength (26%) and rota-rod performance (82%) was observed in rats treated with arsenic (sodium arsenite, 20 mg/kg body weight/day orally, 28 days). A 22-year-old man with chronic arsenical dermatosis died from arsenic-related effects after lifetime exposure to an estimated average dose of 0.014 mg As/kg/day in the drinking water (Zaldívar et al., 1981). Nain and Smits (2012) reported that clinical changes in the rats are limited to decreased feed and water intake in the high (40 ppm) dose group (P < 0.05), however, growth rate was not affected.

There were many case reports of death in humans due to ingestion of high doses of arsenic. In nearly all cases, the most immediate effects were vomiting, diarrhea, and gastrointestinal hemorrhage, and death may ensue from fluid loss and circulatory collapse (Saady et al., 1989; Uede and Furukawa, 2003).

5.2 Body weight

Body weight is commonly used as an indicator for evaluating general health. In the present investigation arsenic intoxication significantly decreased the body weight in mice from day 7th to day 30 (p< 0.001) during total experimental period as compared to control animals (DDW treated).

A 41-year old woman exposed to arsenic in the drinking water for 4 months at an approximate dose of 0.06 mg As/kg/day reported losing 40 pounds (18 kg) of body weight before seeking treatment (Wagner et al., 1979). Weight loss was also among the effects observed in a series of 475
chronic arsenism patients hospitalized in Antofagasto, Chile after receiving approximate doses of 0.02 mg As/kg/day in the drinking water for an unspecified number of years (Zaldivar, 1974). Feed consumption and body weight gain were significantly reduced in a dose-related manner in dogs fed 1.5 or 1.9 mg As/kg/day as sodium arsenite in the diet. The effect on body weight was due to reduced feed consumption, rather than a direct effect of arsenic (Neiger and Osweiler, 1989).

Male rats exposed to 5.0 ppm arsine for 28 days showed decrease in body weight gain (Blair et al., 1990). Rats given a single oral dose of 100 mg As/kg as GaAs exhibited a 15% reduction in body weight compared to controls 7 days after exposure (Flora et al., 1998). High doses of arsenic showed decreased body weight in mice and rabbits (Nemec et al., 1998). Reductions in body weight gain are commonly seen in animal studies of ingested arsenic. In pregnant rats, body weight gain was reduced by gavage treatment with 23 mg As/kg/day as arsenic trioxide on day 9 of gestation (Stump et al., 1999).

Two pregnant mice that died after repeated gavage treatment with 24 mg As/kg/day as arsenic acid had hemorrhagic lesions in the stomach (Nemec et al., 1998). Gross gastrointestinal lesions (stomach adhesions, eroded luminal epithelium in the stomach) were seen frequently in rats treated by gavage with 8 mg As/kg/day as arsenic trioxide starting before mating and continuing through the end of gestation (Holson et al., 2000). People in Bangladesh and in West Bengal were clinically examined in arsenic-affected districts. Apart from generalized weakness, weight loss was observed in patients (Rahman et al., 2001).

Exposure of rats by gavage to 26.6 mg As/kg/day as sodium arsenite for 4 weeks resulted in a significant decrease in body weight (Schulz et al., 2002). The decreases in body weight gain occurred at doses that were
associated with diarrhea and histological alterations in the gastrointestinal tract (Arnold et al., 2003).

In a 12-week oral gavage study, rats dosed with 1.5 mg/kg/day sodium arsenite had a median final body weight 18% lower than controls (Dhar et al., 2005). Oral administration of arsenic trioxide for 30 days caused dose dependent significant reduction in body weight (Verma et al., 2004).

A 60-day rat study with sodium arsenite in the drinking water reported a 13% reduction in final body weight in rats dosed with approximately 0.02 mg As/kg/day (Bashir et al., 2006). Poor body weight in arsenic exposed rats due to oral exposure to arsenic for a period of 12 weeks significantly (P<0.05) increased arsenic burden in blood, liver and kidney from arsenic treated rats. Arsenic treated rats had comparatively poor body weight gain over time, and the mean body weights of these rats were significantly (P<0.05) lower from 10th weeks onwards (Nandi et al., 2006).

Islam et al. (2001) and Liu et al. (2006) reported that mice exposed to arsenic showed loss in body weight.

Children treated low arsenic weighted more than those in the high arsenic treated groups (Wang et al., 2007). Sarkar et al. (2012) also reported that arsenic exposure can affect children's growth. Maharjan et al. (2007) conducted a survey in an area of lowl and Nepal observed that arsenic intake was negatively correlated with body mass index and substantially increased prevalence of underweight individuals.

Treatment with sodium arsenite indicated decreased body weight in mice (Sharma et al., 2007 (4mg/kg b.wt); Sharma and Kumar, 2011 and in rats El-Demerdash et al., 2009 (5mg/kg b.wt); Dwivedi and Flora, 2011; Aliyu et al., 2012).
Inorganic arsenic could significantly decreased body weight gain compared to control (Pari and Mohammed Jalaludeen, 2011). Tsang et al. (2012) reported that utero-exposure of arsenic (0 and 85 ppm as arsenite) caused further significant body weight reduction in mice foetuses.

Messarah et al. (2012) reported that exposure of arsenic (5.55 mg/kg b.w/day) for three consecutive weeks in rats, resulted significant decrease in body weight gain (−13%) as compared to the control.

Sarkar et al. (2012) reported that a gradual weight loss in arsenic treated group of mice was observed indicating arsenic mediated growth retardation.

Bhattacharya and Haldar (2012a) revealed the body weight of rats from toxin control group (after 8 days) were significantly ($p < 0.001$) decreased when compared with normal control group.

A study by Singh et al. (2013) observed exposure to arsenic in mice caused a significant decrease in body weight (-19%) as compared to control and could be associated with decreased food consumption and water intake. Subchronic toxicity of Inorganic arsenic caused to decrease in gain of body weight gain compared to control (Jiang et al., 2013).

Increased permeability of intestinal lining may be responsible for improper absorption of nutrients due to toxicant induced malnutrition and loss of appetite in animals. The cause of death is massive fluid loss due to secretion from the gastrointestinal tract eventuating in severe dehydration, reduced circulating blood volume, and consequent circulatory collapse. On postmortem examination oesophagitis, gastritis, and hepatic steatosis were reported (Ratnaike, 2003).

It is revealed that arsenic and its metabolites are absorbed through intestinal lining when it is permeable and affect the gastrointestinal tract
and causes gut leaky. If the liver gets more arsenic beyond its detoxifying capacity and cannot remove toxicants, they can recirculate to the intestinal area where they increase the permeability of the intestinal lining even more (Nutriwest, 2003).

5.3 Liver Weight

In the present study arsenic intoxication produced significant (P<0.001) decrease in the liver weight throughout the 30 day experimental period. Male rats exposed to a time-weighted average (TWA) dose of 72.4 mg MMA (Monomethyl arsenite)/kg/day for 104 weeks showed a decrease in absolute liver weight, while females exposed to 98.5 mg MMA/kg/day showed histiocytic proliferation of the liver (Arnold et al., 2003).

Oral administration of arsenic trioxide at the dose of 3 and 6 mg/kg b. wt./day) for 30 days showed dose dependent reductions in liver weight (Verma et al., 2004).

Sharma et al. (2007) revealed that Swiss albino mice were treated with arsenic as sodium arsenite NaAsO$_2$ (4 mg/kg b.wt.) intraperitoneally in 0.9% NaCl. A reduced liver weight was reported significantly in arsenic treated group.

Raikwar et al. (2008) reported liver weight loss after arsenic intoxication in livestock. Arsenic toxicity caused a change in the balance between cell death and proliferation coupled with a robust loss of liver weight with hepatocyte loss caused by cell death (Arteel et al., 2008).

Sharma and Kumar (2011) reported a significant reduction in liver weight after arsenic intoxication (4 mg/kg b.wt.) in mice for 30days.

Generalized icterus (Jaundice) was reported in dogs after acute exposure to roxarsone (an arsenic compound). Some small fluctuations in liver weight have been noted in rats and mice after intermediate oral exposure to roxarsone (ATSDR, 2011).
Bhattacharya and Haldar (2012a) revealed liver weight of rats of arsenic (10 mg/kg b.wt.) intoxicated group (after 8 days), were significantly ($p < 0.001$) decreased when compared with normal control group.

In a study by Bhattacharya and Haldar (2012b) was done in arsenic (10 mg/kg b.wt.) induced toxicity in male Wistar albino rats. The liver weights of rats from arsenic treated group (after 10 days) were significantly ($p<0.001$) decreased as compared to normal control group.

Chandranayagam et al. (2013) reported the change in general parameters such as body weight, feed intake and organ weight (liver) in the arsenic-induced experimental rats for 28 days. The growth was comparatively reduced in arsenic-intoxicated rats when compared to the normal rats.

Owumi et al. (2013) reported that sodium arsenite (2.5 mg/kg b. wt.) administration in rats showed a significant decrease in liver and relative liver weights of arsenic treated rats as compared to control.

A study by Singh et al. (2013) observed exposure to arsenic in mice caused a significant decrease in liver weight (-25%) as compared to control and could be associated with decreased food consumption and water intake. Szinicz and Froth (1988) have also reported reduction in glycogen concentration during arsenic poisoning. Marked depletion of carbohydrate mainly glucose was reported to be a major problem in acute arsenic poisoning leads to reduction in liver weight. Arsenic is known to inhibit growth by interfering with various metabolic processes (Cong and Ma, 2002).

5.4. Histopathological aspects

In the present investigation arsenic administration produced various pathological alterations in liver of Swiss albino mice.
The great susceptibility of the liver to damage is a consequence of its primary role in the metabolism and detoxification of foreign compounds (Brandon et al., 2003) and arsenic accumulates in this organ after exposure (Centeno et al., 2002). A number of studies in humans exposed to inorganic arsenic by the oral route have noted signs or symptoms of hepatic injury. Clinical examination often reveals that the liver is swollen and tender (Liu et al., 2002 b), and analysis of blood sometimes shows elevated levels of hepatic enzymes (Mazumder, 2005). The liver regulates several important metabolic functions and the hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999).

In the present study, Arsenic administration in the Swiss albino mice showed various pathological alteration in liver. Hepatocytes showed cytoplasmic vacuolization and degranulation. Various nuclear changes in hepatocytes such as karyolysis, karyorhexis, hypertrophy and enucleation were also observed in liver of Swiss albino mice. These histopathological alterations was more prominent from day 3 to day 30. Sinusoidal spaces were more expanded. There was an increase in Kupffer cells and binucleated hepatocytes were also observed.

In a study by Gyasi et al. (2012), experimental mice were introduced to 0.8–4.8 mg/L arsenic, Histopathological studies revealed hepatic cell swelling with the loosening of cell wall and degenerative change with cells showing cytoplasmic vacuolation with nuclear blebbing and gradual cell loss. Mice exposed to arsenic showed ultrastructural changes in hepatocytes. Acute reaction was characterized by enlargement of the surfaces of some inner membranous structures of hepatocytes (invaginations of nuclear membrane and undulations of mitochondrial surface) as well as by loss of glycogen. This response is considered as a direct toxic action of arsenic (Mohelska et al., 1980). Acute exposure of monkeys to 6 mg As/kg/day resulted in
vacuolization of the hepatocytes (Heywood and Sortwell, 1979). Naranmandura et al., (2012) reported that rat liver mitochondrial swelling is strongly induced by exposure to the methylated forms of MMA(III) and DMA(III) in a dose-dependent manner in the absence of Ca$^{2+}$, suggesting that the methylated forms may have potent effects on cellular mitochondria. Short-term arsenic exposure (3mg/kg b. wt./day for 30 days) caused liver damage evidenced by activities of liver enzymes and necro-inflammatory changes. These effects of arsenic were coupled with enhanced mitochondrial swelling, inhibition of cytochrome-c oxidase, Ca$^{2+}$-ATPase, a decrease in mitochondrial calcium content, changes in indices of hepatic mitochondrial oxidative stress and iNOS expression. Arsenic also increased hepatic caspase 3 activity and DNA fragmentation (Majumdar et al., 2011).

Liu et al., (2000) also observed that metallothionin-null mice and the corresponding wild type (WT) mice were exposed to arsenite and arsenate through drinking water or through repeated subcutaneous injections, induces damage to liver. Although overt hepatocellular necrosis did not occur, fatty infiltration and liver degeneration (inflammation and focal necrosis) were observed (Mathews et al., 2012).

Arsenic (4.2 mg/kg/b.wt.) exposed mice were exhibited peripancreatic necrosis, necrosis in hepatocytes, nuclear degeneration and appearance of vacuoles. In the case of, multiple dose of sodium arsenate treated mice more severe changes were observed. These changes included nuclear degeneration, cytoplasmic degeneration, emptied portal vein, binucleated condition and appearance of vacuoles in hepatocytes (Devaraju et al., 2010).

In the present study, focal necrosis was observed on day 3. In epidemiology studies of human arsenic exposure (Tong et al., 2001), liver histology showed degenerative liver lesions, such as chronic inflammation, vacuolation and focal necrosis. Arsenic induces cell death in liver. In a study
by Bashir et al. (2006) male Wistar rats were exposed to arsenite at three different doses of 0.05, 2.5 and 5 mg/L for 60 days. Histopathological examination under light and transmission electron microscope suggested a combination of ongoing necrosis and apoptosis. DNA TUNEL showed an increase in apoptotic cells in liver (Javaid et al. 2008; Srivastava et al., 2013).

In a study by Manna et al. (2007) and Muthumani and Milton Prabhu (2013) administration of sodium arsenite at dose of 10 mg/kg body weight for 2 days, murine histology revealed centrilobular necrosis. Histologic assessment revealed macroscopic changes, including more necroinflammatory foci and a more robust infiltration of neutrophils. Furthermore, arsenic exposure expanded the portal areas in the liver (Arteel et al., 2008).

Srivastava et al. (2013) reported that arsenic trioxide induces disorganization of hepatic parenchyma and disruption in the epithelial lining of the central vein and vacuolar degeneration.

It is reported that arsenic intoxication showed liver injury in varying degrees such as hydropic degeneration, fatty degeneration, spotty necrosis, focal necrosis and inflammatory cell infiltration. There are liver cell regeneration and fibroplasia in varying degrees. The liver injury of the mice in all arsenic groups aggravated as exposure time prolonged (Wu et al., 2009).

Histological studies showed well-differentiated signs of focal hepatitis, lobular inflammation, prominent hepatocyte degeneration, and severe periportal necrosis due to arsenic intoxication (Kumar et al., 2010).

Hepatic steatosis with occasional collection of mononuclear inflammatory cells and mild portal fibrosis were the predominant liver lesion observed after 9 months of arsenic exposure, while at 12 months, the changes included mild hepatic steatosis, inflammation, necrosis and significant fibrosis in periportal areas (Mazumder 2005; Ghatak et al., 2011).
Qualitatively, the necrotic changes, decrease in hepatocyte microvilli, degeneration of mitochondria and vacuolar encroachment of nuclei and dilation of sinusoids were observed after lead intoxication in male Wistar rat (Narayana and Al-Bader, 2011) and arsenic intoxication in mice (Vijaya Baskara Reddy et al., 2012).

Hepatic necrosis might be due to oxidative stress induced by arsenic that further involved in cellular protein degradation. The sinusoidal spaces were expanded in our present study may be due to shrinkage and necrosis of hepatic cells (Singh and Rana, 2007).

Swinbourne et al. (2013) reported histopathological changes in the liver of cats such as hepatocyte swelling with microvesicular change, fibrosis, biliary hyperplasia and haemosiderin within Kupffer cells.

Ferreira et al. (2012) reported that significant ultrastructural changes occurred in hepatic cells with evident cellular and cytoplasm disorganization in hepatocytes characterized by an increase in the number of organelles, mainly mitochondria and rough endoplasmic reticulum, organelles that in the case of the exposed mice, are probably responsible for the enzymes’ synthesis that have the function of inactivating the toxic metabolites. Extended vacuolated areas, mainly in regions next to the cell nucleus is observed. Alterations observed in the nuclei of the hepatocytes pointed out cell death processes.

The present study also revealed that the number of Kupffer cells was increased. Kupffer cells are known to participate in the early events of liver injury involving lipid peroxidation. Kupffer cells have metabolic ability to detoxify 4-Hydroxy-2,3-(E)-nonenal (4-HNE), a major aldehydic product of lipid peroxidation (Luckey and Peterson, 2001). Kupffer cells are potentially vulnerable to the increased concentrations of 4-HNE during oxidative stress.
Arsenic Toxicity

- Metabolic Dysfunction
- Free radical production
- In cell injury
- Attachment with SH protein
- Glycogen depletion and Fat droplet Accumulation

- Dilation of sinusoidal and central vein
- Oxidative damage
- Nucleus
- Membrane damage
- Cytoplasmic vacuolization

- Nonspecific Breakdown of DNA
- Nuclearchanges {Pyknosis, Karyortesis, Karyolysis}

Hepatocellular damage

Hepatopathological lesion

A proposed mechanism of Arsenic Toxicity
In a study by Mandal et al. (2007) rats injected with a single dose of sodium arsenite produced hepatic damage. Histopathological examination revealed hepatocellular and fatty metamorphosis, necrosis, Kupffer cell hyperplasia, fibrosis and deposition of collagen contents. Kupffer cells increased in number (hyperplasia) suggesting an increase in the phagocytic activity of the liver in the exposed mice (Ferreira et al., 2012).

The present study showed occurrence of binucleate and quadriplet cells. These were observed in normal hepatic tissue as well as in arsenic treated tissue although the occurrence was more in arsenic treated hepatic tissue.

Grizzi and Chiriva-Internati (2007) stated that occurrence of binucleate hepatocytes may be an index of the severity of hepatic illness. Study by Karapetian and Dzhivanian (2006) shows that post traumatic regeneration of the liver after partial hepatectomy involves activation of DNA synthesis in hepatocytes, leading to increase in their ploids. Regeneration of granular parenchyma of the liver is accompanied by a quantitative increase in binucleate hepatocytes, which is most highly expressed with in 5-20 days after partial hepatectomy.

Most cells in mammalian tissues usually contain a diploid complement of chromosomes. However, numerous studies have demonstrated a major role of “diploid-polyploid conversion” during physiopathological processes in several tissues.

Although adult liver is a quiescent organ, it retains a capacity to proliferate and to modulate its ploidy in response to various stimuli or aggression (partial hepatectomy, metabolic overload (i.e., high copper and iron hepatic levels), oxidative stress, toxic insult, and chronic hepatitis etc.). Gentric et al. (2012) reviewed the mechanisms and functional consequences of hepatocytes polyploidization during normal and pathological liver growth.
Liver show a high percentage of polyploid cells. Thus, during postnatal growth, the liver parenchyma undergoes dramatic changes characterized by gradual polyploidization during which hepatocytes of several ploidy classes emerge as a result of modified cell division cycles. This process generates the successive appearance of tetraploid and octaploid cell classes with one or two nuclei (mononucleated or binucleated). Liver cells polyploidy are generally considered to indicate terminal differentiation and senescence and to lead both to the progressive loss of cell pluripotency and a markedly decreased replication capacity. In adults, liver polyploidization is differentially regulated upon loss of liver mass and liver damage. Interestingly, partial hepatotomy induces marked cell proliferation followed by an increase in liver ploidy (Celton-Morizur et al., 2010).

In rat treated with toxin (biological active substance (BAS) “Trepel” and “Suvar”), the numbers of the binucleated and mitotically dividing hepatocytes is increased, while amount of hepatocytes with dystrophic changes is reduced (Romanova and Malysheva, 2011).

Feussner et al. (1979) suggested that arsenic has direct toxic effect on DNA synthesis that results in marked disturbances in nuclear division.

Gentric et al. (2012) reported that cytokinesis failure events induces the genesis of binucleated tetraploid liver cells.

**Consequences for hepatocytes polyploidization**

1. Polyploidy could protect hepatocytes of genotoxic damage by increasing the number of copies of functional genes; this might be especially important for the liver that has a primary function in metabolizing and eliminating toxic compounds.
2. Polyploidy could be an economical solution to growth problems that occur when an organ work within its capabilities, avoiding the great demand in energy that represents cell division.

3. Finally, polyploidy could alter the expression profile of specific genes.

Anatskaya and Vinogradov studies (2010) showed alteration in a wide range of functional gene groups between diploid and polyploidy hepatocytes. The authors suggested a link between genome multiplication and emergence of specific pathways (increase in metabolic plasticity and for the protection of replicating DNA from oxidative damage) that could promote hepatocyte cell survival and tissue regeneration under stressful conditions.

Arsenic causes oxidative stress that leads to hepatocytes damage

In the present study marked pathological changes were observed in the nucleus of hepatocytes which may be due to oxidative stress caused by arsenic by generating reactive oxygen species (ROS).

When both humans and animals are exposed to arsenic, they experience an increased formation of ROS/RNS, including peroxyl radicals (ROO•), the superoxide radical, singlet oxygen, hydroxyl radical (OH•), hydrogen peroxide, the dimethylarsenic radical, the dimethylarsenic peroxyl radical and/or oxidant-induced DNA damage. Yamanaka et al. (1990) suggested that peroxyl radical play a major role in DNA damage. Arsenic induces the formation of oxidized lipids of which aldehydes [malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE)] are the major end products (Jomova et al., 2011).

It is reported that metal mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation and altered calcium and sulphydryl homeostasis (Valko et al., 2005).
Bhadauria and Flora (2007) reported that inorganic arsenic (25 ppm for 10 weeks) administration shows oxidative stress induced DNA damage. Ding et al. (2005) reported that trivalent and pentavalent arsenic exposure induces oxidative DNA damages (8-OHdG) by the involvement of ROS and RNS generation in human keratinocytes.

In a study inorganic arsenic treatment to primary cell culture of β-cells and hepatocytes in vitro produced cyto-degenerative effect and accumulated reactive oxygen species (ROS) in pancreatic β-cells and hepatocytes of mice (Chakraborty et al., 2012).

On the day 15th in arsenic treated group, arsenic intoxication causes cytoplasmic vacuoles, frothing and nuclear condensation with the intact membrane, indicating the possible induction of apoptosis whereas higher dose of arsenic leaded to the appearance of necrosis (Yang et al., 2003). The \( \text{As}_{(2)}\text{O}_{(3)} \) exposure promoted extensive DNA damage and apoptosis resulted suppression of antioxidant system (including GPx) and promotion of ROS formation, increase of mitochondrial membrane potential damage, DNA damage, and caspase-3 activity elevated expression of p53, cytochrome c and Bax proteins levels. (Biswas et al., 2010; Selvaraj et al. 2013).

Qu et al. (2008) reported that oxidative stress and inhibition of DNA damage repair is the main potential carcinogenic action of arsenic (Martinez et al. 2011). It also inhibit the function of a key DNA repair protein, PARP-1 (Proline Rich Acidic Protein-1), which appears to protect cell from apoptosis by inducing cell-cycle arrest (Komissarova and Rossman, 2010) even at very low concentration, thus exacerbating the overall oxidative DNA damage.
5.5 Biochemical aspects

(a) Sodium arsenite and lipid peroxidation

Lipid peroxidation is one of the measures to determine cellular toxicity. It is a complicated chain reaction leading to formation of a variety of degradation products (Bandyopadhyay et al., 1999). Among these products, malonaldehyde (MDA) is often used as a reliable marker of lipid peroxidation (Fang et al., 2002) and degree of peroxidative damage to cell membrane can be assessed (Cini et al., 1994). The measurement of byproducts of lipid peroxidation and status of antioxidant enzymes are appropriate ways to assess the prooxidant-antioxidant status in tissues. MDA can react with the free amino group of proteins, phospholipids or nucleic acids to produce inter and intra molecular 1-amino-3-imino propene (AIP) bridges and structural modifications of biological molecules (Halliwell and Gutteridge, 1985).
Lipids have a critical structural and functional role in membranes. Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and low density lipoproteins (Deckers, 1996). PUFAs allows fluidity of cellular membranes. Any disruption of this role can lead to cell death. The carbon-carbon double bonds found in polyunsaturated fatty acids are ready targets for free radical attack (Kehrer, 2000). A free radical steals electron from the lipid membrane of the cell. This leads to series of reactions resulting in the formation of newly arranged molecules, conjugated dienes (CD). The CD reacts easily with the oxygen to form a peroxyl radical which further carries out chain propagation (Karlsson, 1997). Arsenic generates reactive oxygen species (ROS), such as superoxide (O$_2^-$), hydroxyl (OH) and peroxyl (ROO) radicals (Shi et al., 2004). The elevated free radical concentration decreases the antioxidant defenses in the biological system and thus induces lipid peroxidation. According to Jomova and Valko (2011), along with MDA, 4-Hydroxynonenal (4-HNE) are also formed after arsenic exposure which is a major aldehyde product of lipid peroxidation, contributed significantly to the cytopathological events observed during oxidative stress in the erythrocytes of exposed rats. 4-HNE triggered death signal cascade was initiated with the formation of HNE-protein adducts in cytosol. HNE-protein adduct formation resulted in depletion of cytosolic antioxidants followed by increased generation of ROS.

The present study revealed a highly significant increase in the lipid per-oxidation (LPO) level in sodium arsenite (4.0 mg/kg b.wt.) treated group as compared to control.

In a study done by Boulikas (1991), reactive oxygen species are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic. Free radicals have been detected in some cells treated with arsenite (Liu et al., 2001; Pourhmad et al., 2003).
Sharmilla *et al.* (2009) exposed wistar albino rats to As (III) in drinking water (100 ppm for 4 months). They observed a significant increase in LPO content in liver and kidney.

In a recent study by Banerjee *et al.* (2009) repeated injection of arsenic trioxide induce oxidative stress and hepatotoxicity in mice revealed from elevated levels of lipid peroxidation. In vitro tests of oxidative damage to basic cellular constituents, lipids, proteins, and nucleic acids, were measured using thiobarbituric acid reacting substances (TBARS) assays, protein carbonyl formation and 8-hydroxydeoxyguanosine (8-OHdG), respectively (Nain and Smits, 2012).

Flora *et al.* (2011) suggested that Arsenic showed a significant increase in the levels of reactive oxygen species formation and lipid peroxidation and decrease in the activities of antioxidant enzymes.

Metal-mediated formation of free radicals causes various modifications to nucleic acids, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis (Corradi and Mutti, 2011). Flora *et al.* (2011) reported that significant increase in blood, liver and kidney reactive oxygen species accompanied by an increase in lipid peroxidation points to the involvement of oxidative stress in gallium arsenide toxicity. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders (Alzheimer's disease, Parkinson's disease), chronic inflammation and others (Jomova and Valko, 2011).

Chattopadyay *et al.* (2011) reported significantly increased MDA and CD content in liver tissue homogenates in the sodium arsenite-treated group.
Arsenic is known to induce oxidative damage in the cells by producing lipid peroxidation and protein oxidation, which represent stronger indices of oxidative stress (Hegazy and Ghaleb, 2011). Fouad et al. (2012) reported that sodium arsenite (10mg/kg/day, orally for two consecutive days) treatment increase the lipid peroxidation.

(b) Arsenic toxicity and reduced glutathione

GSH, a tripeptide L-γ-glutamyl-L-cysteinyl-glycine, is the major nonprotein thiol in the body (Miester and Anderson, 1984). The exceptional γ–peptidic linkage is thought to protect the tripeptide from degradation by aminopeptidase.

\[
\text{HOOC-CH-CH_2-CH-NH-CH-C-NH-CH_2-COOH}
\]

\[
\text{NH_2} \quad \text{CH_2} \quad \text{SH}
\]

It is a major component of cellular antioxidant system and acts as a nucleophilic scavenger of numerous compounds and their metabolites via enzymatic and chemical mechanisms and plays important roles in the protection against oxidative damage caused by ROS. GSH has a role in signal transduction (Arrigo, 1999) gene expression (Sen et al., 1999) and apoptosis (Voehringer, 1999). GSH depletion can impair a cell's defense against the toxic actions of many compounds and leads to cell injury and death (Pi et al., 2002).

In the present study, a significant decrease in hepatic GSH content was recorded in arsenic treated mice at various autopsy intervals.

Mazumder (2001) recorded data on liver involvement following chronic exposure to arsenic - contaminated water. They reported studies in patients who consumed arsenic contaminated water for one to 15 years. Initial biochemical evidence of hepatic membrane damage, probably due to reduction
of glutathione was seen by 6 months. Delayed manifestation that follows pro
to antioxidant imbalance.

In case of chronic exposure generally increase in ROS and depletion of
GSH stabilizes and oxidative stress is predominantly expressed as increased
lipid peroxidation, a sign of cell injury (Shi et al., 2004).

Sharma et al. (2007 and 2009) reported that sodium arsenite intoxication
(4 mg/kg b.wt. intraperitoneally in 0.9% NaCl) in Swiss albino mice, showed
significant decrease in GSH activity and a significant increase in LPO content
in liver.

Bhahduria and Flora (2007) also observed adult an increase in hepatic
and renal levels of thiobarbituric acid reactive substances while decrease in
GSH content in adult male Wistar rats following 25ppm arsenic administration
for 10 weeks.

In a study by Das et al. (2010b) reported that arsenite exposure
increased the levels of reactive oxygen species (ROS), arsenic accumulation
and lipid peroxidation and decreased the activities of the antioxidant enzymes
and glutathione in the liver and kidney.

Arsenic alleviated the higher dose-mediated lipid peroxidation and
 glutathione depletion (Manimaran et al., 2010).

Pari and Mohamed Jalaludeen (2011) reported that non-enzymatic
antioxidant like reduced glutathione level is reduced along with increased
elevation of lipid peroxidative markers, thiobarbituric acid reactive substances,
lipid hydroperoxides, protein carbonyl content and conjugated dienes.
Mathews et al. (2012) and Chattopadhyay et al. (2012) reported that arsenic
exposure decreased hepatic superoxide dismutase (SOD), catalase activities
and the level of nonprotein-soluble thiol (NPSH) including GSH, with a
concomitant increase in thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CDs) in the liver in rat.

Arsenic binds with sulfhydryl groups of proteins and various enzymes and interferes with metabolism of GSH that serves as an essential antioxidant molecule responsible for metabolism and excretion of xenobiotics (Hultberg et al., 2001). Enhanced production of free radicals and inhibition of antioxidant enzymes have been suggested as possible mechanisms to explain arsenic induced oxidative damage (Liu et al., 2001).

As (III) and As (V) have been shown to react with GSH to form As-GSH complexes (Percy and Gailer, 2008). Thus depletion in GSH causes reduction in activities of GSH-dependent enzymes.

Thiols are considered to be the first line of cellular defense against arsenic-mediated oxidative damage. Remarkable decrease in the levels of total thiols and GSH has been observed in arsenic-exposed mice that may be due to the binding of arsenic with the various intracellular sulfhydryl groups (Sinha et al., 2008).

Das et al. (2005) reported that the oxidative stress due to arsenic-induced free radicals could cause a dramatic fall in GSH and loss of protective thiols.

The fact that arsenic decreases GSH levels has been observed in liver of rats and mice over time (Sharma et al., 2009; Yadav et al., 2012).

Several pathways by which arsenic can deplete cellular GSH levels have been proposed. First, GSH could act as the electron donor for the conversion of pentavalent arsenicals into the trivalent arsenicals (Radabaugh and Aposhian, 2000). Second, arsenite has a high affinity for GSH. Third, free radicals induced by arsenic may oxidize GSH (Ramanathan et al., 2003).
Notably, the arsenite-mediated depletion of GSH levels may further enhance the oxidative damage induced by arsenite, because it is known that mammalian cells use GSH to remove excess ROS (as a result, the intracellular concentrations of GSH in mammalian cells are inversely correlated with the degree of oxidative stress) (Rosenblat and Aviram, 1998).

It is also possible that arsenic may be indirectly toxic through the disruption of cellular methylation reactions (Zhao et al., 1997). In biological systems, inorganic arsenic exists as either arsenate (As$^{5+}$) or arsenite (As$^{3+}$) (Aposhian, 1989). Inorganic arsenic is metabolized in many cells, via enzymatic methylation, to monomethylarsonic (MMA) and dimethylarsinic acids (DMA) (Abernathy et al., 1999). Generally speaking, methylated arsenicals are far less reactive than the inorganic forms of arsenic, a fact which may account for their being far less acutely toxic in biological systems. Also, methylated arsenicals are more readily eliminated via the urine. For these reasons, methylation of inorganic arsenic is often thought to be a mechanism by which arsenic is detoxicated. Methylation of arsenic also consumes glutathione (GSH) and S-adenosyl-methionine (SAM) (Zakharyan et al., 1995) and therefore, arsenic toxicity may partially result from the depletion of the cellular pools of either GSH or SAM (Shimizu et al., 1998).

GSH depletion may be responsible for accumulation of free radicals that initiate lipid peroxidation resulting in biochemical damage after covalent binding to biomacromolecules. GSH also protects the membrane polyunsaturated fatty acids from peroxidation and has an antioxidant function. When oxidative stress generated reactive oxygen species (ROS) exceeds the capacity of antioxidant defense to counteract ROS or the antioxidant capability of the cells deteriorates, the cellular damage is produced (Flora, 2011).
(c) **Arsenic toxicity and Antioxidant enzymes - Superoxide Dismutase (SOD), Catalase (CAT) & Glutathione Peroxidase (GPx)**

Generation of highly reactive oxygen species (ROS) is an integral feature of normal cellular function. Small amount of potentially toxic reactive oxygen species (ROS) could be generated in eukaryotic cells by normal oxidase action and during the course of electron transport in mitochondria or endoplasmic reticulum (Halliwell and Gutteridge, 1990; Forman and Boveris, 1992). ROS includes hydrogen peroxide superoxide anion hydroxyl radical and peroxynitrite (ONOO-). They are produced as byproduct of mitochondrial respiration or oxidase such as nicotine adenine diphosphate (NADPH) oxidase, xanthine oxidase (XO) and certain arachidonic acid oxygenases (Zorov et al., 2006). Their production however, multiplies several folds during pathological conditions.

A change in the redox state of the tissue implies a change in ROS generation or metabolism, their rich coordination chemistry and redox properties are such that they are capable of escaping out of the control mechanisms such as homeostasis, transport, compartmentalization and binding to the designed tissue and cell constituents. Breakdown of these mechanism can lead to the metal binding to protein sites other than those tailors for that purpose or displacement of other metals from their natural binding sites thus causing oxidative deterioration of the biological macromolecules (Valko et al., 2005).

**Generation of reactive oxygen species (ROS), beyond the body's endogenous antioxidant balance cause a severe imbalance of the cellular antioxidant defense mechanism (Sinha et al., 2010).**

SOD and CAT are two important radical scavenging enzyme and body’s secondary defense against oxygen metabolites produced due to toxic metals/metalloid. SOD, Glutathione peroxidase and CAT play an important
role in the detoxification of reactive oxygen species such as \( \text{O}_2^- \), OH, and H\(_2\)O\(_2\), which are involved in genotoxicity (Oberley and Oberley, 1986) and produced due to toxic metals/metalloid. Thus, these enzymes activities help in maintaining the physiological level of oxygen and hydrogen peroxide by dismutation of oxygen radicals and decomposition of hydrogen peroxide (Gonzales et al., 1984) and hence offer a protective role against free radicals.

![Figure 1: Mechanism of anti-oxidant enzymes in which SOD neutralizes the superoxide anions converts into H\(_2\)O\(_2\) that eliminated by catalase and GPx (Glutathione peroxidase). GPx consumed GSH into GSSG. GSSG converted into GSH by using NADPH in presence of GSH reductase.](image)

**SOD** is considered to be a stress protein, which is synthesized in response to oxidative stress. SOD has been detected in large number of tissues and organism and is thought that it is present to protect the cell from damage caused by \( \text{O}_2^- \) (Fridovich, 1972). Accumulation of \( \text{O}_2^- \) and H\(_2\)O\(_2\) results in the production of \( \cdot \text{OH} \) by a metal-catalysed reaction. Therefore, SOD is reported to inhibit \( \cdot \text{OH} \) production (Fridovich, 1973). It catalyzes the dismutation of superoxide to H\(_2\)O\(_2\) and these forms are degraded by catalase and glutathione peroxidase (Mc Cord et al., 1971; Usoh et al., 2005).

Superoxide dismutase converts \( \text{O}_2^- \) to H\(_2\)O\(_2\) according to the following reaction:

\[
\text{SOD} \quad \text{O}_2^- + \text{O}_2^- \rightarrow 2 \text{H} \quad \text{H}_2\text{O}_2 + \text{O}_2
\]
The transition metal of the enzyme reacts with $\text{O}_2^{•−}$ taking its electron. $\text{O}_2^{•−}$ is the only known substrate for SOD. There are different types of SOD such as Cu-Zn-SOD is found in the cytosol of most eukaryotic cells. A different form of Cu-Zn-SOD is found in extracellular fluids, where it is called EC-SOD (Marklund, 1984). Mn-SOD is located in the mitochondrial matrix and bacteria, while Fe-SOD is present in many aerobic bacteria. Each type of SOD has its own peculiarities; however, all types of the enzyme have similar properties (Oberley and Oberley, 1986).

In the present study the decreased SOD activity in liver suggested that accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following arsenic treatment (Shi et al., 2004). Arsenic significantly ($p<0.01$) reduced mRNA expression of the superoxide dismutase 2 (SOD2) gene in male Wistar rats (Rana et al., 2012).

**Catalase** (CAT) is the second enzyme which acts in cellular detoxification. It is a tetrameric enzyme consisting of four identical tetrahedral arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit and has a molecular mass of about 240 kDa (Aebi, 1980). Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen.

The reaction of catalase in the decomposition of hydrogen peroxide is:

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

Catalase reacts very efficiently with $\text{H}_2\text{O}_2$ to form water and molecular oxygen besides this it also react with H donors (Methanol, Ethenol, Formic acid and Phenols) with peroxide activity.

There are a large number of sources of $\text{H}_2\text{O}_2$ during aerobic growth of an organism. The major damage from $\text{H}_2\text{O}_2$ arises from the highly reactive
hydroxyl (OH\(^{•}\)) radical, generated in the Fenton reaction between H\(_2\)O\(_2\) and metal ions such as Fe\(^{2+}\), which can react with various components of cells, making it desirable for most aerobic organisms to have catalases or peroxidases to circumvent the damage.

Catalase protects cells from the reactive oxygen species that are generated within them due to arsenic (Manna et al., 2007).

In present investigation Catalase level were found to be minimum in arsenic intoxicated group as compared to control group.

In rats exposed to arsenic, the possible accumulation of H\(_2\)O\(_2\) in organs as a result of diminished activity of catalase is probably circumvented by the increased GSH concentrations stimulating glutathione peroxidase mediated reduction of H\(_2\)O\(_2\) and organic peroxides (Yu, 1994).

Exposure of BALB/C mice to 0.7 mg arsenic/kg/day in the drinking water for 15 months resulted in changes in liver enzymes (glutathione S-transferase, glutathione reductase, catalase and glutathione peroxidase) (Santra et al., 2000).

Reduced activity of catalase is reported in previous studies by arsenic intoxication in male Wistar rat (Gopalkrishnan and Rao, 2006; Bashir et al., 2006).
Glutathione peroxidase (Gpx) is a GSH consuming antioxidant defense enzyme. It is selenium dependant enzyme, converts H$_2$O$_2$ into water via the oxidation of reduced glutathione to oxidized glutathione (GSSG). Glutathione peroxidase exists in soluble form associated with the membrane as phospholipid hydroperoxide glutathione peroxidase and acts on lipid hydroperoxide (De Leve and Kaplowitz, 1991).

Gpx reduces lipid peroxides into lipid alcohols in the presence of GSH. The decrease in the level of GSH and increase in lipid peroxidation causes decrease in the activity of Gpx during arsenic exposure (Wang et al., 1997).

In the present investigation Gpx activity was significantly reduced in arsenic intoxicated group as compared to control.

As Gpx is a selenoenzymes, arsenic affects the synthesis and activity of selenoenzymes, it may directly interact with Se (Selenium) and form insoluble and inactive As–Se complex (Jain et al., 2011) resulting in the inhibition of Gpx activity. Messarah et al. (2012) reported that exposure of rats to arsenic caused a significant increase in liver TBARS. The activities of glutathione peroxidase (Gpx) and glutathione-S-transferase (GST) of arsenic-treated group were found lower.

Thus in present study, The levels of all the three antioxidants showed significant decrease.

A decrease in SOD activity increases the level of O$_2^-$, which is known to inactivate catalase activity (Kono and Fridovich, 1982); when catalase or Gpx fails to eliminate H$_2$O$_2$ from the cell, the accumulated H$_2$O$_2$ causes inactivation of SOD (Sinet and Garber, 1989).

A decrease in the activity of SOD can be attributed to an enhanced superoxide production during arsenic metabolism (Flora et al., 2002). Gupta and Flora (2005a) demonstrated that arsenic treatment (0.2mg[sol]/kg,
intraperitoneally) showed significantly decreased activities of SOD in rats. Arsenic treatment (10 ppm in drinking water) associated with decreased activities of SOD and CAT was observed in liver and kidney of rats (Nandi et al., 2006).

The study demonstrated a significant decrease in hepatic superoxide dismutase and catalase activities at the different doses of arsenic (10.5 & 12.6 mg/kg of b. wt.) in Wistar rats (Bashir et al., 2006).

Arsenic (Sodium arsenite III) produces excessive ROS production that causes to decrease in the activity of antioxidant enzymes such as SOD, CAT and GPx in human lymphocytes (Sinha et al., 2007) and in brain, hepatic and renal tissues in rats (Modi and Flora, 2007).

Manna et al. (2007), Sinha et al. (2008) and Chowdhary et al. (2008) revealed that antioxidant enzyme showed a decrease in hepatic and renal SOD, CAT and GPx activities in all arsenic-induced mice groups and Sprague-Dawley rats.

Banerjee et al. (2009) and Flora et al. (2009) reported that reduction in hepatic SOD, CAT and GPx activities were observed after repeated injection of arsenic trioxide in mice.

The rats exposed to sodium arsenite at a dose of 10 mg/kg body weight, exhibited a significant inhibition (p< 0.01) in hepatic and renal antioxidant enzymes namely SOD, CAT, GPx, GST and GR (Das et al., 2010b).

Sinha et al. (2010), Biswas et al. (2010) and Kumar et al. (2010) reported that arsenic depletes antioxidant enzymes activities like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and glutathione (GSH) in mice liver tissue.
Chattopadyay *et al.* (2011) reported a dramatic decrease in hepatic SOD and CAT activities in all arsenic-induced female Albino rats groups when compared to the normal control group ($P<0.001$). Male wistar rats were chronically exposed to sodium arsenite for eight months, the results demonstrated a significant decrease in hepatic GSH levels, SOD and catalase activities and an increase in TBARS levels after arsenic administration (Jain *et al.*, 2011).

The toxic effect of arsenic was also indicated by significantly decreased activities of enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and glucose-6-phosphate dehydrogenase along with nonenzymatic antioxidants like reduced glutathione, total sulfhydryl groups (Muthumani and Prabu, 2012; Chakraborty *et al.*, 2012).

### (d) Arsenic Toxicity alters ATPase

ATPase forms a large family of membrane proteins which couple ATP hydrolysis to the active transport of cations or other compounds such as phospholipids across cell membranes (Lutkenko and Kaplan, 1995; Moller *et al.*, 1996) and is also considered as a master enzyme that controls many important functions at cellular and organ level including active transport and electric potential across plasma membrane, intracellular pH regulation, cell division and cell elongation (Serrano, 1989).

In the present study ATPase activity in liver was significantly decreased in arsenic intoxicated group at all autopsy intervals.

The increased activity of LPO is correlated with the decreased activity of ATPase, since LPO induces the breakdown of membrane as the free radical plus intermediate products of LPO are capable of damaging the integrity and altering the function of biomembrane (Subramanian *et al.*, 1994; Mazumder, 2001).
The membrane bound ATPase $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{Na}^{+}/\text{K}^{+}$ ATPase regulate the movement of ions (Axelson and Palmson, 1998). As cofactors, these ions are involved in mitochondrial and cytological enzyme reactions (Petris, 2004). Nonsignificant decrease under stress may be due to the adaptive changes occupying inside the body. Decreased activity of enzyme ATPase may lead to decrease in ATP breakdown, leading to reduction in the free energy and thus creating a disturbance in ion homeostasis (Vorbrot et al., 1994). Difnis et al. (1992) reported that heavy metal binds to SH-groups of ATPase leading membrane disorganisation finally inhibiting ATPase activity (Vujusic et al., 2004).

Arsenic disrupts ATP production through several mechanisms. Arsenic (III) decreased the hepatic concentration of GSH and ATP in a dose dependent manner, but increased the level of S-adenosyl methionine. After GSH depletion the retained arsenic (III) can increasingly inhibit SH-enzymes, thus causing ATP depletion and energetic disorder (Csakany et al., 2003). At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NADH, mitochondrial respiration, and ATP synthesis. (Klaassen and Watkins, 2003).

![Figure 2: Arsenic inhibits ATP generation through aerobic respiration by blocking pyruvate dehydrogenase (PDH) that converts pyruvate into acetyl co-enzyme A.](Cited by Google)
Arsenic treatment led to disturbed ADP:O ratio, abnormally decreased ATPase activity in liver of rats (Singh and Rana, 2010).

Muthumani and Prabu (2012) reported that the toxic effect of arsenic is indicated by significantly decreased activities of hepatic membrane bound ATPases in rats.

In a study by Flora et al. (2012) observed a significant decrease in the activity of ATPase in the liver after arsenic exposure.

(e) Lactate Dehydrogenase (LDH) and Arsenic Toxicity

Lactate Dehydrogenase (LDH) is a cytoplasmic marker enzyme (Kim et al., 2001).

![LDH Reaction]  

It catalyses the inter conversion of lactate and pyruvate in the glycolytic activity. In most toxicological studies, LDH release is a convenient screening method for assessment of irreversible cellular injury (Lash, 1989). The LDH release of cells is an index of their viability. It is a sign of integrity of plasma membrane since the LDH activity outside the cell measured, high LDH release is a sign for a low viability (Danpure, 1984).

In the present investigation, there was a significant decrease in LDH activity following arsenic intoxication in liver of Swiss albino mice. Decrease was more when hepatocyte were completely damaged.

Ayala-Fierro et al. (2000) investigated that arsenic directly inhibits LDH activity. Toxicity in isolated cells was determined by LDH leakage and
LDH release in serum occurs only when cells have ruptured, an indicative of cell death. The cytotoxicity of trivalent and pentavalent inorganic arsenic salts was determined in mouse fibroblasts in vitro. Increase in arsenic (III) and arsenic (V) concentrations led to an increase in LDH release and reduction in cell proliferation (Fischer et al., 1985 and 1989).

The degeneration of cytoplasm with the appearance of large vacuoles and loss of cell-cell junction were observed. Biochemically determined increase in the viability of L-LDH showed an increased in extra-cellular release (Yang et al., 2003). The results showed that hepatocyte viability significantly decreased in dose dependant manner in heavy metal treated group (Wang et al., 2006).

Significant increase in LDH activity was observed with increasing concentrations of arsenic in water, hair and nails. LDH activity was found to be increased in the higher exposure groups of water and hair arsenic concentrations. LDH activity was also increased at low to medium exposure groups of nail arsenic concentrations. Thus, the elevated plasma LDH activity might be helpful for the early prognosis of organ or tissue damage in the individuals who were exposed to arsenic chronically (Karim et al., 2010).

Human, rat and porcine (Pigs) hepatic cell culture were treated with sodium arsenite. It was reported to release LDH from the cell due to arsenic intoxication but the release is highest in human hepatic cell (Langsch et al., 2009).

A study done by Xu et al. (2009) reported the releasing out of LDH from C6 cells in cell culture caused apoptosis and necrosis in that cells.

As$_2$O$_3$ induced the decrease of intracellular GSH, increase of ROS and increased trypan-blue-positive cells, the release of LDH, apoptosis, ROS and loss of mitochondrial membrane potential (Cheng et al., 2010).
Yoshino et al. (2011) reported that a significant dose dependant arsenic (III)-mediated cytotoxicity was observed in the primary cultured chorion and amnion cells prepared from human fetal membranes accompanied with an increase of LDH release.

Wang et al. (2012b) reported that LDH release was increased after arsenic intoxication in cardiac H9c2 cells. Wang et al.(2013b) showed that arsenic increased the apoptosis and necrosis of H9c2 cardiomyocytes by generation of ROS.

(f) **Liver Function Tests**

To evaluate the liver function following parameters were done in serum of Swiss albino mice.

(i) **Serum Glutamate Oxaloacetate Transaminase (SGOT)/ Aspartate Aminotransferase (AST) and Serum Glutamate Pyruvate Transaminase (SGPT)/ Alanine Aminotransferase (ALT) (Aminotraferases)**

In the present study, a significant increase in serum AST and ALT activity at day 7, 15 and 30, clearly indicates liver damage due to arsenic toxicity.

Liver is an important organ for metabolism and detoxification and its functional integrity is determined by serum-AST and ALT enzyme activity. For practical purposes, ALT and AST are currently widely employed as potential markers of hepatocellular toxicity.

Arsenic is known to produce disturbances in liver function (Fowler et al., 1977). Mazumder et al. (1998) performed a study on large number of people encountered with arsenic toxicity due to drinking of arsenic contaminated water in 6 districts of West Bengal. Clinical and various laboratory investigations were carried out on 156 patients to ascertain the
nature and degree of morbidity and mortality that occurred due to chronic arsenic toxicity. Liver function test showed elevated ALT in 11.8% and AST in 27.6% cases.

SGOT and SGPT are reliable determinants of liver parenchymal injury (Moss et al., 1987). The increment of the activities of SGOT and SGPT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of arsenic.

Elevated levels of serum aspartate aminotransferase (AST) were observed in rats administered a single oral dose of 100 mg As/kg as gallium arsenide (Flora et al., 1998).

The murine model, relevant to epidemic human toxicity in areas of arsenic concentration, showed that continued arsenic feeding resulted in fatty liver with serum ALT, AST elevated at 12 months and hepatic fibrosis at 15 months (Santra et al., 2000).

Liu et al. (2002) reported 4 to 6 fold increase in serum alanine aminotransferase activity. Serum alanine amino transferase activity increased after 24 hours of sodium arsenite (12-19 mg/kg) exposure in mice.

In a study by Flora et al., (2002) Gallium arsenide exposure was responsible for increased serum aminotransferase moderately in rats.

Borgognone et al. (2005) also observed that release of cytosolic enzyme, alanine aminotransferase following arsenic exposure in isolated rat hepatocytes.

Gupta and Flora, (2005a) demonstrated that arsenic treatment (0.2mg/kg, intraperitoneally) showed significantly increased activities of ALT and AST in rats.
In a study by Modi et al. (2006) male Wistar rats were exposed to arsenic as sodium arsenite (2 mg/kg, orally through gastric intubation). Exposure to arsenic produced a significant elevation of serum AST & ALT activities.

Sinha et al. (2007) demonstrated the effect of taurine against sodium arsenite in murine hepatocytes. Arsenic treatment caused an increase in the activities of serum ALT that confirmed membrane damage due to toxin exposure.

In an investigation by Modi and Flora (2007) and Diwedi and Flora (2011) mice were exposed to arsenic (25 ppm in drinking water for 6 months. Hepatic AST and ALT activity decreased significantly on arsenic exposure. Because of liver injury, these enzymes leaked-out of liver into peripheral blood.

The studies examined subchronic exposure via drinking water to low doses of a mixture of metals (Jadhav et al., 2007) including arsenic (Singh and Rana, 2007). Changes in blood clinical markers were observed.

In the study Manna et al. (2007) and Dalal et al. (2009) reported that the serum marker enzymes aminotranferase increased in the arsenic treated group by 75% compared to the normal control.

Following hepatocellular injury, there will be moderate to marked increase in both serum ALT & AST (Modi et al., 2006; Ramaiah, 2007).

Sharma et al. (2007 and 2009) observed that sodium arsenite (4 mg/kg body weight) intoxication caused a significant elevation in serum aminotranferases level in Swiss albino mice.

Banerjee et al. (2009) demonstrated that repeated injections of arsenic trioxide induced oxidative stress and hepatotoxicity in mice as revealed from elevated levels of SGOT and SGPT.
Mohan Chandrasekaran et al. (2010) investigated the toxic doses of arsenite increased serum aspartate and alanine transaminase levels and lipid peroxidation in liver tissue in rats.

Rana et al. (2010) and Islam et al. (2011) elucidated that there is significant elevation of plasma (serum) enzyme activities of both alanine aminotransaminase and aspartate aminotransaminase due to higher arsenic burden in Zebu cattle which are consumed arsenic contaminated water chronically. With regards to the exposure-response relationship with arsenic in the drinking water, the respective activities of ALP, AST and ALT were found to be significantly increased in the high-exposure compared to the lowest exposure groups.

Chattopadyay et al. (2011) reported that sodium arsenite (0.4 ppm /100 gm b.wt./day) administration caused significantly increased enzymatic activities compared to the normal control group (P<0.001).

Activities of both SGOT and SGPT were significantly higher in arsenic treated mice indicating liver dysfunction. There was 16.67% increase in SGPT level and 20.59% increase in SGOT level of arsenic treated mice as compared to control (Yasmin et al., 2011).

Histopathology showed damage in arsenite - and arsenate-treated liver which is corroborated by increased serum alanine aminotransferase (ALT) levels, indicative of liver injury (Liu et al., 2011).

Arsenic exposure significantly increased (p<0.05) the activities of plasma alanine aminotransferase-glutamate pyruvate transaminase (ALT/GPT) and aspartate aminotransferase-glutamate oxaloacetate transaminase (AST/GOT). In contrast, the mitotic index in these cells is significantly reduced (p<0.05). These findings indicate that aminotransferases are biomarkers for arsenic induced hepatotoxicity (Patlolla et al., 2012).
Patra et al. (2012) reported that significantly elevated serum alanine aminotransferase and aspartate aminotransferase activities (p<0.05) along with histopathological changes in liver indicated arsenic induced hepatotoxicity in goat following long term oral exposure. Messarah et al., (2012) reported that a significant increase in plasmatic activities of AST, ALT and ALP is observed in arsenic-treated group of rats.

(ii) Serum Alkaline Phosphatase

The present study suggests that NaAsO₂ treatment significantly increase the serum alkaline phosphatase (ALP) activity throughout the experimental period i.e. from day 1 to day 30.

Phosphatases (AcP and ALP) are important and critical enzymes in biological processes, they are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function (Yousef et al., 2008). Alkaline Phosphatase are a family of zinc metaloenzymes with a serine at the active center; they release inorganic phosphate from various organic orthophosphate. Alkaline Phosphatase is located histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes (Rosalki and McIntyre, 1999). This enzyme has a membrane location (Lanca and Israel, 1991). Highest levels of alkaline phosphates occur in cholestatic disorders. The leakage from bile canaliculi into hepatic sinusoidal may result from leaky tight junction (Kaplan, 1986; Rosalki and McIntyre, 1999). Damage to cell membrane caused by NaAsO₂ may be one of the reasons for increase in activity of serum alkaline phosphatase. The damage to cell membrane, leads to the imbalance between synthesis and degradation of enzyme proteins (Hardonk and Koudstall, 1976).
In a study by Sharma et al. (2007) and Bayramoglu et al. (2011) suggested that the increase in the activities of these enzymes in plasma and the decrease in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, and this showing the stress condition of the treated animals. Also, they reported that the increase in the activity of these enzymes in blood might be due to the necrosis of liver and kidney.

Hernandez-Zavala et al. (1998) studied liver function in subjects from three towns of Lagunera Region in Mexico. Mean Arsenic concentration in drinking water in towns ranged from 14 to 300 lg/l. Significant increased rate of serum alkaline phosphatase and total bilirubin was noted in subjects from the town with highest exposure in contrast to the town with lowest exposure (Hernandez-Zavala et al., 1998; NRC, 2001).

Arsenic treatment (0.2mg/kg, intraperitoneally) showed significantly increased activities of hepatic ALP in rats (Gupta and Flora, 2005a). The serum marker enzymes ALP increased in the arsenic treated mice group by 68% compared to the normal control (Manna et al., 2007).

Sinha et al. (2007) demonstrated that arsenic treatment in murine hepatocytes caused increase in the activities of serum ALP that correlated with membrane damage. Treatment with Sodium arsenite caused significant increase in the activities of ALP in plasma and decrease in liver and this may be due to the leakage of these enzymes to the blood stream (Yousef et al., 2008).

Repeated injections of arsenic trioxide induced oxidative stress and hepatotoxicity in mice as revealed from elevated levels of alkaline phosphatases (Banerjee et al., 2009). Individuals chronically exposed to arsenic via drinking water showed predominantly increased serum ALP activity, suggesting the presence of cholestasis (Hernandez-Zavala et al., 1998).
5.6 Modulation of arsenic toxicity by *Chlorophytum borivilianum*

Dietary antioxidants in the form of nutrients appear to be of great importance in controlling damage by free radicals. Naturally occurring vitamins, minerals, phytochemicals and synthetic compounds are determined to be safe and effective without causing any undesirable toxicity (Greenwald et al., 2002; Chandranayagam *et al.*, 2013), however, each nutrient is unique in terms of its structure and its particular antioxidant function. Phytochemical substances in plants and plant derived foods such as *Chlorophytum borivilianum* (*C.borivilianum*) have gained growing attention. They are also being widely investigated for their antioxidant activity and other possible health-promoting potentials (Thakur *et al.*, 2009).

In the present study, *C. borivilianum* root extract antioxidant potential was observed by DPPH radical scavenging activity. Kaur *et al.* (2010) also reported that *C. borivilianum* have a significant DPPH radical scavenging activity (52.83% at 1000 µg/ml) in *in vitro* study.

Many *in vitro* and *in vivo* studies have shown that *C. borivilianum* possess beneficial effects against oxidative stress related diseases by directly scavenging free radicals (Oudhia, 2001) and by increasing the activities of antioxidant enzymes (Manna *et al.*, 2007).

*C. borivilianum* root extract have many phytoconstituents such as saponins (Bordia *et al.*, 1995), simple sugars (Sreevidya *et al.*, 2006) fructooligosaccharides (Visavadiya and Narasimhacharya, 2007) proteins, phenolics, gallotannins and triterpenoids and mucilage (Thakur and Dixit, 2005).

In the present study in combination groups (*C. borivilianum* (800 mg/kg b. wt.) with sodium arsenite (4.0 mg/kg b. wt.)) did not show any skin lesion, sickness and ruffled hair, this may be due to immunomodulatory (Govindrajjan *et al.*, 2005) and antioxidant properties of root extract (Kaur *et al.*, 2010).
There had been reports for the presence of specific polysaccharides in *C. borivilianum*, exhibit biological activity to improve the health. Perhaps, polysaccharides would lead to the production of nutritional supplements and will stimulate the immune system (Paulsen *et al.*, 2001 & 2002). The enhanced responsiveness is indicative to upregulating the macrophages, dendritic cells and lymphocytes in rats (Fulzele *et al.*, 2002).

*C. borivilianum* root extract acts via a cascade of mechanisms modulating the immune system and to restore the health. *C. borivilianum* have potency to enhance phagocytic activity and removal of foreign particle/xenobiotics (Thakur *et al.*, 2009).

Present investigation revealed significant enhancement in body and liver weight in combination groups IV&V (*C. borivilianum* with sodium arsenite) during 30 day experimental period as compared to arsenic intoxicated group (III). Arsenic cause disturbances in the gastrointestinal tract and erodes the gut epithelium including brush border and makes the gut leaky (Ratnaike, 2003) because of that poor absorption of nutrient/food occurs which cause loss in body weight gain.

Jackson *et al.* (1999) reported that *Chlorophytum* species extract rendered significant protection against gastrointestinal tract ulcer. In stress induced ulceration model, proved antioxidant activity was observed where it reversed the lipid peroxidation. Thus modulates impair of the digestion of protein and uptake of vitamins and minerals in the gut (Francis *et al.*, 2002). Kenjale *et al.* (2007) reported that ulcer index is reduced by *C. borivilianum* in rats.

As *C. borivilianum* is rich with saponins, Johnson *et al.* (1986) found that some saponins increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances. Thus in the present study it can be suggested that saponins present in *C.*
*borivilianum* protected gut epithelium from the toxic effect of arsenic, increases nutrient absorption which causes gain of body weight.

Arsenic causes ROS production and oxidative stress and can cause injury to hepatocytes. Liver cells have a particularly high probability of being subjected to ROS induced toxicity because of detoxification of xenobiotics and toxic substance. Oxidative stress appears to be involved in the mechanism of various types of cell injury (Stoh, 1995).

In the present study in both the combination groups, liver histopathology showed modulation as evident by less cytoplasmic vacuolization, less widening of sinusoids and no karyorhexis, karyolysis and focal necrosis at day 30. Central vein was not occluded. Necrosis which is severe form of injury was observed on day 30 in arsenic treated group was prevented and minimized in the present study.

Lee *et al.* (2004) reported that hepato-histopathological alterations such as degeneration of hepatocytes and hepatic cords, focal necrosis, karyolysis, karyorhexis and congestion in central vein sinusoids and infiltration were prevented after treatment of saponins.

Histological studies by Manna *et al.* (2007) revealed less centrilobular necrosis in liver treated with saponin (arjunolic acid) alongwith arsenic in mice. In the present study, cytoplasmic vacuolization/degranulation was observed from day 3 to 30 which is due to the glycogen depletion. Higher concentration of arsenic causes glycogenolysis in liver (Haque *et al*., 2009).

Granulated cytoplasm was observed in combination groups. It is possibly ameliorated by *C. borivilianum* root extract as it is rich with simple sugars like glucose, fructose, xylose, galactose, mannose (Sreevidya *et al*., 2006).
Thus the present study suggested that *C. borivilianum* reduced the incidence of liver lesion inflammation including hepatocyte shrinkage, karyolysis, karyorhexis, leucocytes infiltration and necrosis by blocking cytotoxicity induced by arsenic and decreasing the progressive damage and exerts an antioxidant action inside the cells and it is responsible to observed modulation of the cellular response to oxidative challenge.

Khanal *et al.* (2009) reported that saponins significantly prevented the increased hepatic TNF-α level, hepatic lipid peroxidation and hepatic triglyceride level along with steaosis and necrosis in liver.

Li *et al.* (2010) reported that Saponin *Panax japonicus* (SPJ) plays an important role in the protection of the structure and function of hepatic mitochondria and karyon and reduction in hepatic cord degeneration, focal necrosis and vascular congestion.

It has been reported that arsenic causes hepatocyte damage by stimulating apoptotic pathway (Flora *et al*., 2009). But *C. borivilianum* shows the modulation in hepatocyte by revealing its cytoprotective effect as it is rich with several phyto-constituents along with saponins. Ghosh *et al*., (2010b) reported that saponin mitigates the dysfunction by setting an equilibrium between pro and anti apoptotic members of Bcl-2 family of proteins as well as reduced the mitochondrial membranes potential, reduced the concentration of cytochrome c and caspase-3 activity. Li *et al*., (2010) also reported that saponin significantly attenuated the TUNEL-positive cell and reduced the expression of caspase-1 and caspase-3.

Cytotoxicity is possible interference with the detoxication process that cause the depletion of GSH, which can indirectly lead to cell integrity and DNA damage and result in cell injury (Halliwell and Gutteridge, 1999). A relationship between GSH concentration and the extent of hepatocyte injury has been established. The present study showed that arsenic reduced the level
Discussion

of GSH, an index of oxidative stress in liver and that treatment with *C. borivilianum* significantly prevented the arsenic induced depletion of GSH. The formation of lipid peroxidation products and depletion of GSH implicate oxidative stress. Present study reported that combined administration of *C. borivilianum* with sodium arsenite resulted in significant reduction in level of LPO and increase in GSH content in liver of Swiss albino mice as compared to arsenic intoxicated group (III).

**Therefore the results showed that *C. borivilianum* attenuated the oxidative stress induced by arsenic.**

Lipid solubility of arsenic and enhanced lipid peroxidation disrupts membranes biophysical properties. As *C. borivilianum* is rich with saponins, they protect the biomembranes. Saponins interacts with the polar heads of membrane phospholipids which will result in their later ability to form micelle-like aggregates. Moreover, their hydrophobic aglycone backbone could intercalate into the hydrophobic interior of the bilayer. Both of these effects may contribute to the alteration of the lipid environment around membrane proteins. It has become increasingly evident that the lipid environment of membrane proteins, including ion channels, transporters, and receptors, plays an important role in their function. The changed-function of proteins or glycoproteins in the plasma membranes have been suggested to be the cause of secondary biochemical responses induced by saponins (Abe *et al*., 1978; Rao and Sung, 1995). A study by Fu *et al.* (2009) that saponins have antioxidant effect by inhibition capabilities of lipid peroxidation and also prevent the depletion of glutathione level as arsenic reduces GSH (Khanal *et al*., 2009).

The protective effect of *C. borivilianum* on arsenic induced oxidative hepatotoxicity could be associated with inhibition of organic hydroperoxide to alkoxy radical that subsequently initiated lipid peroxidation. Manna *et al.*
(2007) reported that saponins prevent the enhancement of DNA fragmentation, protein carbonyl content, lipid peroxidation end product (MDA) and level of oxidized glutathione.

Sharma and Kumar (2011) also reported the significant reduction in LPO level and increased GSH content by *C. borivilianum* treatment in arsenic induced toxicity in liver of mice.

In the present study increased SOD, CAT and Gpx activities were observed during total 30 day experimental period in both combination groups IV & V (*C. borivilianum* + arsenic + *C. borivilianum* & arsenic+ *C. borivilianum*) as compared to the arsenic treated group. Increased activities of these enzymes are due to antioxidant potential of *C. borivilianum*, as the level of these antioxidants are maintained histoarchitecture of liver also show modulation in terms of less damage. This can be correlated with different constituents present in *C. borivilianum* especially saponins.

*In vitro* study done by Sreevidya et al. (2006) reported that *C. borivilianum* root extract have potent superoxide radical inhibition property perhaps due to the presence of saponins in root extract.

In a study, a group of saponins (group B soyasaponins) produced in legumes allows saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing bio-molecular damage by free radicals (Yoshiki and Okubo, 1995; Yoshiki et al., 1998; Hu et al., 2002). Further these hydroperoxide are neutralized by glutathione peroxidase (GPx) that consumes GSH (Messrrrah et al., 2012).

Manna et al. (2007) reported that arjunolic acid (A saponins)(at dose of 20 mg/kg b.wt.) treatment prior to arsenic intoxication showed increased activities of hepatic antioxidant enzymes SOD, CAT, GST, GR and GPx as well as increased the reduced glutathione and total thiol content compared to arsenic treated group.
Saponins significantly reduced DNA damage and increased SOD, CAT and GPx activities in bone marrow cells and peripheral lymphocyte cells (Quing et al., 2009). GSH content are also restored. Saponin can inhibit free radical damage by inducing the transcription of SOD, GPx and CAT gene (Qu et al., 2012). Increased expression of SOD is helpful to induce CAT activity because $\text{H}_2\text{O}_2$ is an inducer of CAT (Manna et al., 2007). Saponins could increase CAT activity that inhibited by arsenic, which can help to detoxifying local increasing $\text{H}_2\text{O}_2$ concentration. Parkes et al. (1998) reported that insertion of extra copies of the SOD gene into various genome has produced transgenic animals with highly improved resistance to oxidative stress.

Ye et al. (2008) reported that prospective of saponins against toxicant induced cytotoxicity, ascribed to its antioxidant properties by reducing intracellular reactive oxygen species level, enhancing the activities of enzymes SOD and GPx. Mitochondrial membrane potential could be stabilized.

Because of antioxidant property, *C. borivilianum* can directly scavange ROS/free radical and upregulate the expression of antioxidant enzyme (SOD, CAT and GPx). Li et al. (2010) also reported the same in mice.

The hepatic enzymes AST, ALT and ALP are used as biochemical marker for hepatic damage. LDH is used as an index of cell viability. In the present study the increased activities of AST, ALT and ALP had been detected in serum in arsenic treated mice, implying the increased permeability, damage and necrosis of hepatocytes.

Exposure to arsenic had been shown to increase activity of liver transaminases in the blood (Chattopadhyay et al., 2001). This is an index of hepatotoxicity which might have resulted from oxidative stress-related damages to hepatocytes membrane and leakage of hepatic transaminases into extracellular spaces ultimately finding their way into the blood from the liver (Owumi et al., 2013).
The decreased LDH activities in hepatocytes were observed in combined treatment groups compared to arsenic treated group.

Present study also showed a significant decrease in the activities of AST, ALT and ALP in the serum in combination groups (IV & V that is \textit{C.borivilianum}) as compared to arsenic treated group. This can be attributed to be the antioxidants present in \textit{C. borivilianum}.

Manna \textit{et al.} (2007) revealed that combined administration of arjunolic acid with sodium arsenite reduced the level of serum marker enzymes, alanine aminotransferase and alkaline phosphatase as compared to arsenic administered mice (Qu \textit{et al.}, 2012).

Khanal \textit{et al.} (2009) reported that Changkil saponins significantly prevented the increased serum alanine aminotransferase activity, hepatic lipid peroxidation and prevent the depletion of glutathione level.

Present study suggest that pre and post treatment of \textit{C. borivilianum} maintains GSH content, decrease lipid peroxidation, exhibited the ability to quench free radicals as observed by DPPH assay, protected the cells effectively from injury caused by arsenic, as reflected in the increased cell viability in term of increased LDH activity in hepatic tissue and deceased activities of serum AST, ALT and ALP thus maintained the level of AST, ALT and ALP.

ATP are required for proper functioning of all ATPases. The present study reported that in combination groups, total ATPase activity was significantly increased as compared to arsenic treated groupIII.

Arsenic depletes ATP the energy currency (Csanaky \textit{et al.}, 2003). It also dissipates electrochemical gradient across membranes including mitochondrial functioning (Majumdar \textit{et al.}, 2011) that led to disturbance of ionic equilibrium and energy status of hepatocytes.
The increased membrane damage and ATP depletion induced by arsenic were abolished using *C. borivilianum*. It is reported that cholesterol enrichment of membrane have an inhibitory effect on many membrane ATPases, as it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein. Ginsenosides (from *Panax quinquefolius* and *Panax japonicus*) share the steroid backbone and amphipathic nature with cholesterol (Yamasaki *et al.*, 1987; Choi *et al.*, 2001). It is possible that some ginsenosides interact with membrane cholesterol and displace it from the immediate environment of ATPases. Since removal of cholesterol will lead to an increase in membrane fluidity, conformational changes that ATPases undergo during their transport cycle may be facilitated. Membrane fluidity controls the enzyme activity of biological membranes and has an important role in ion transport (Ma and Xiao, 1998) and the ability of saponins to affect this parameter may explain their effects on cellular function.

*C. borivilianum* treatment have potential to mitigate all biochemical and histopathological alterations after arsenic intoxication. Our finding from liver histopathology suggests ameliorative potentials of *C. borivilianum* on arsenic induced hepatocytes damage. This is in accordance with some recent studies that recommend the use of antioxidants and antioxidant-rich foods and herbal medicinal plant for the management of arsenicosis (Chandranayagam *et al.*, 2013). **Thus it may be concluded that active constituents found in *Chlorophytum borivilianum* decreases the oxidative stress, quenches free radicals and induces free radical scavenging enzyme activity and thus reduces the arsenic induced cellular injury in liver.**