Summary and Conclusions
Introduction

Arsenic is one of the most toxic metals derived from natural environment. It is ubiquitously present in the ecosystem in both organic and inorganic forms. Inorganic arsenic causes major human concern. Arsenic can enter the body via inhalation or consuming arsenic contaminated food and drinking water or through skin contact (Rahman, 2012; Erraguntla et al., 2012). Episodes of arsenic poisoning caused by drinking ground water have already reported in various countries and parts of the world especially South Asia including Bangladesh and Eastern India (Rahman et al., 2009). Arsenic concentration in drinking water is 10ug/L is safe (Bundschuh et al., 2012). The chronic arsenic exposure has been linked with a myriad of possible health effects, including skin lesions, hypertension, cardiovascular disease, reproductive and neurological dysfunctions, hematological changes and malignancies of skin and internal organs (Gopalkrishnan and Rao, 2006; Santra et al., 2007).

After absorption, inorganic arsenic is accumulated in the liver, spleen, kidneys, lungs and gastrointestinal tract. Liver is considered as the primary target for toxicopathological manifestations of arsenic (Sharma and Kumar 2011). In many animals including mammals, inorganic arsenic is metabolized in the liver. During metabolism, most of the inorganic arsenic such as As (III) and As (V) are metabolized to dimethylarsinic acid and monomethylarsonic acid and then rapidly cleared from tissues through urine (Watanabe and Hirano, 2012).

Oxidative stress due to accelerated production of free radicals has been implicated for arsenic-caused injury in liver (Flora, 2011). The liver possesses an antioxidant defense system that removes peroxides, free radicals and superoxide anion generated within the cell such as GSH, SOD and catalase.
GSH is a critical component of this defense system. It promotes arsenic methylation by stabilizing the redox state of the cell (Percy and Gailer, 2008). SOD is considered to be a stress protein, which is synthesized in response to oxidative stress. 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. But arsenic overrides these antioxidant system.

**Natural products and their active principles as sources for new drug discovery and treatment of diseases** have attracted attention in recent years (Newman and Cragg, 2007). Trends on applying nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years (Saha and Khuda-Bukhsh, 2013). To fight against arsenic intoxication (Which is responsible for oxidative insult in metabolic machinery) the present study was designed to find out a suitable ameliorative factor of arsenic toxicity.

*Chlorophytum borivilianum* (Safed musli) belongs to the family Liliaceae, is a medicinal herb, and is native to the tropical and subtropical regions of Africa and Asia (Kaushik, 2005). It is found in the oldest mountain ranges on the continent, the Aravalis (Rajasthan) India (Thakur and Dixit, 2006). It is a small perennial rhizomatous herb. Rhyzomes are short and inconspicuous while roots are usually thicker or fleshy (Marais and Reilly, 1978). It is a rich source of over 25 alkaloids, saponins, polyphenol, tannins, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose (Thakur *et al*., 2009). Its roots (tubers) are widely used for various therapeutic applications. Anticancer (Deore and Khadabadi, 2010), immunomodulatory (Thakur *et al*., 2009), anti-diabetic (Govindrajan *et al*., 2005), antistress (Kenjale *et al*., 2007), analgesic (Panda *et al*., 2007), anti-inflammatory
Antioxidant (Kaur et al., 2010) and antibacterial (Sundaram et al., 2011) activities of root extracts have been evaluated.

The present study was planned to evaluate the effect of root extract of *Chlorophytum borivilianum* on arsenic induced hepato-pathological alteration and the possible alleviation of arsenic induced toxicity in Swiss albino mice.

**Materials and Methods**

**Animals:** Healthy male Swiss albino mice (*Mus musculus*), 6-8 weeks old (average body weight 22 ± 2 gm), maintained under controlled conditions of temperature and light (light - 14 hrs. darkness - 10 hrs.) were used for present study. These animals were provided standard mice feed (obtained from Hindustan Limited, Delhi) and water *ad libitum*.

**Preparation Chlorophytum borivilianum root extract:** The roots were air dried in shade and powdered. The powder was distilled in soxhlet apparatus for 36 hrs using DDW (Double Distilled Water) at 40°C. The distilled extract was dried in oven at 36°C and redissolved in DDW before administration. The dose of Cb root extract was selected on the basis of minimum LPO and maximum GSH that is 800mg/kg b.wt.

**Toxicant:** Arsenic in the form of sodium arsenite (trivalent), NaAsO$_2$ was used for the present study. It was obtained from HiMedia India Limited, Mumbai. It was dissolved in DDW and administered orally 4 mg/kg b.wt./day.

*Chlorophytum borivilianum* root extract was given to mice at different dose levels (100, 200, 400, 800 mg/kg b.wt.) by oral gavage for 7 days consecutively. Out of these doses, 800 mg /kg b.wt. was selected for experimental protocol.
Parameters studied
The following parameters were assessed in the present investigation:

General parameters
i. Mortality
ii. Sickness
iii. Body weight changes
iv. Liver weight changes

Histopathological and Biochemical Parameters
A. Liver
i. Histopathological alterations
ii. Lipid Peroxidation (Ohkawa et al., 1979)
iii. Glutathione estimation (Moron et al., 1979)
iv. Total ATPase (Sickevitz and Potter, 1953)
v. Superoxide dismutase (Marklund and Marklund, 1974)
vi. Catalase (Aebi 1984)
vii. Glutathione peroxidase (Paglia and Valentine, 1967)
viii. Lactate dehydrogenase (Wroblewski, 1987)

B. Liver Function Tests
i. Serum Glutamate Oxaloacetate Transaminase (Reitman and Frankel, 1957)
ii. Serum Glutamate Pyruvate Transaminase (Reitman and Frankel, 1957)
iii. Serum Alkaline Phosphatase (Kind and King, 1954)

Results
General Parameters
* Any sign of toxicity and sickness was not observed in the animals treated with different doses of Chlorophytum borivilianum root
extract i.e. 100, 200, 400 and 800 mg/kg b.wt. orally for 7 days consecutively.

♦ From the various doses given, the dose of 800 mg/kg b.wt. of *Chlorophytum borivilianum* root extract was selected for experimental study on the basis of maximum GSH content and low LPO level was observed at this dose.

♦ Arsenic (4 mg/kg b.wt./day) in form of NaAsO$_2$ was given for 30 days orally. Mortality was not observed in this group but animals showed some sickness in term of hair loss.

♦ Significant increase in body weight was noticed in *Chlorophytum borivilianum* root extract (800 mg/kg b.wt.) alone treated animals as compared to control (only DDW treated) group. In NaAsO$_2$ treated group loss in body weight was significant. In post treatment group no significant increase in body weight was observed. In combination (pre & post treatment) groups a significant gain in body weight were observed as compared to arsenic treated group.

♦ Alterations in liver weight were observed nonsignificant in *Chlorophytum borivilianum* root extract alone treated animals group. In arsenic treated group a significant decrease in liver weight was observed. In Combination (post-treatment) group no significant increase in liver weight was observed. In combination (pre & post) group a significant increase in liver weight were observed as compared to arsenic treated group.

Histopathological studies

♦ When *Chlorophytum borivilianum* root extract alone was given, any pathological alterations were not observed during whole experimental period. Normal histoarchitecture of liver was observed.
There were various pathological alterations in hepatocytes i.e. karyolysis, karyorhexis, focal necrosis, hypertrophy, enucleation along with occluded central vein, widening of sinusoidal spaces, cytoplasmic vacuolization and degranulation in arsenic treated group.

The combined administration of *Chlorophytum borivilianum* root extract and arsenic (Pre and post treatment) showed significant modulation of hepatic damages caused by arsenic.

Post administration of *Chlorophytum borivilianum* root extract with arsenic showed reparation of arsenic induced toxicity from day 1 to day 30.

**Biochemical observations**

i. **Arsenic treated group (as compared to DDW control)**
   - A significant *increase* was observed in MDA level in liver and SGOT, SGPT and alkaline phosphatase in serum of mice as compared to control.
   - A significant *decrease* was observed in hepatic GSH content, LDH activity and total ATPase as compared to control (DDW).
   - The activities of hepatic antioxidant enzymes SOD, CAT and GPx were significantly *decreased* as compared to control.

ii. **Chlorophytum borivilianum** root extract treated Group (as compared to control i.e. DDW)
   - A significant *decrease* was observed in MDA level in liver and SGOT, SGPT and alkaline phosphatase in serum of mice as compared to control.
   - **Hepatic GSH** content was maintained in liver with respect to control.
♦ A significant increase was observed in hepatic LDH and ATPase activities in mice as compared to control.

♦ No significant alterations were observed in SGOT, SGPT and alkaline phosphatase activity in serum of mice as compared to control.

♦ A significant increase was observed in the activities of hepatic antioxidants SOD, CAT and GPx of mice, as compared to control.

iii. Combination groups: *Chlorophytum borivilianum* root extract + Arsenic + *Chlorophytum borivilianum* root extract and Arsenic + *Chlorophytum borivilianum* root extract

♦ The combined treatment groups of *Chlorophytum borivilianum* root extract with arsenic (Pre and post treatment) and (Post administration of *Chlorophytum borivilianum* root extract with arsenic treatment) significantly modulated various biochemical parameters in the liver such as:-

- MDA content was significantly reduced in liver of mice as compared to arsenic intoxicated mice.

- A significant elevation was observed in LDH, ATPase activities and GSH content in liver of mice as compared to arsenic intoxicated mice.

- SGOT, SGPT and alkaline phosphatase in serum were significantly decreased in mice as compared to arsenic treated mice.

- Activities of hepatic SOD, CAT and GPx were significantly increased as compared to control.
Conclusions

On the basis above results, following conclusions were made:

- **Arsenic exerts toxicity through generation of reactive oxygen species through lipid preoxidation mainly and decreases GSH level which results in biochemical alterations.**
- The decreased activities of total ATPase is resulted owing to degradation of membranes.
- Decreased activity of LDH in hepatic tissue is a sign of cytotoxicity of arsenic. Releasing out of LDH from cells indicate cell death.
- A significant increase was observed in the activities of hepatic SOD, CAT and GPx of mice, due to less adverse effect of reactive oxygen species on its functional structure. It may be due to modulatory effect of *Chlorophytum borivilianum*.
- SGOT, SGPT and serum ALP are liver function marker enzymes in serum. Arsenic treatment caused a significant increase in the activities of SGOT, SGPT and serum ALP owing to hepatocyte membrane destruction which may lead to the cells spilling out the enzymes into the blood.

Due to various phytoconstituents including saponins in *Chlorophytum borivilianum* root extract modulates arsenic toxicity by scavenging free radicals and alleviation of hepatohistoarchitecture.

Thus, from the present study it is recommended that *Chlorophytum borivilianum* root extract can be used to a major extent against arsenic toxicity.