Espoused by the Hippocrates nearly 2500 years ago, has renewed interest. The nature has provided valuable medicinal plants which imparted to foods a health giving and curative functions. The uses of herbal remedies have been used for centuries and continue in use in many parts of the world today. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing lifestyle related disorders (Agyare et al., 2009). However, relatively very little knowledge is available about their mode of action and safety. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to world health organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individual’s from developed countries use herbal medicine, which has compounds derived from medicinal plants. Utilizing the healing properties of plants is an ancient practice. People in all continents have long used thousands of indigenous plants for treatment of various ailments dating back to prehistory. Therefore such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998).

Considering the above facts in mind the present study on two medicinal plants viz., Oroxyllum indicum (L.) Kurz and Meyna spinosa Roxb. ex Link. occurring throughout northeastern India was taken up as these plants could be new potent sources of antioxidants because these plant materials are widely used in treatment of diabetes mellitus and other ailments by several ethnic groups of northeastern India for centuries and also of the fact that no proper scientific investigation was carried out on these plants.

HERBAL REMEDIES GLOBAL PERSPECTIVE:

The use of medicinal plants all over the world leads to the introduction of other modern drugs and antibiotics. The first one to use herbal medicines (Greek) was Hippocrates, who uses garlic for herbal remedies (Rivlin, 2001). Herbal medicine became an integral part of primary health care and has been widely used over the various countries, like China, Ethiopia, Argentina and Papua New Guinea (Akinyemi et al., 2005). Other countries, like, Australia, Africa and America have also used medicinal
plants for various herbal remedies, even without having a well recorded history (Amusan et al., 2002; Desmarchelier et al., 1996; Hart, 1981).

Interestingly, the demand for herbal remedies is increasing day by day in industrialized countries because of its effective interventions and therapeutic effect for various diseases and its symptoms (Abere et al., 2010). According to the World Health Organisation (WHO) about almost 80% of world’s developing countries fulfil their primary needs by using traditional medicine (Abere et al., 2010; Calixto, 2000; Green, 2000; Jadeja et al., 2011). About 25% of prescribed and dispensed drugs in United States have atleast one active component or some are synthesized to mimic the active component by the use of plant extracts and it have been seen that about 1/3 rd natives of United States had tried one of this medicine form (Eisenberg et al., 1993). It is reported that the use of alternative therapies by using plant sources increased from 33.8% in 1990 to 42.7% in 1997 (Eisenberg, 1997).

In the last few decades, many research had been carried out to study the intrinsic worth of traditional medicine with the aim to adopt effectively beneficial plants with biological and pharmacological properties and discouraging the harmful ones (Abere et al., 2010). Note worthily, various components of botanical origins serve as starting materials for almost about 25% of pharmaceutical drugs. For example, the tree pacific yew tree yields the breast-cancer fighting drug taxol (tamoxifen). And the herbs, foxglove and salicin are the source for digitalis and aspirin, respectively. However, sometimes the patients may get affected by the possible hazards from these herbal drugs, by lack of manufacturing standards and regulatory agencies oversight (Eisenberg et al., 1998).

Now days, the analysis of plant products which is used enormously because of their extra-ordinary health benefits has been highly growing for identifying the concentration and purity of the active ingredient that shows therapeutic effect and also for testing the amount of active components which could cause lethal toxic effects. It is for this reason the hydroalcoholic stem bark extract and leaves extract of O. indicum and M. spinosa, respectively were considered to evaluate the safety and toxicity in the present study.
SAFETY EVALUATION OF MEDICINAL PLANTS:

Drugs in use need to be safe at the recommended dose. Therefore, before approval of the drugs for use, in most of the countries the safety of the drugs needs to be certified by regulatory authorities. If there is any kind of toxicity in the drugs, it gets banned in the market and its circulation is stopped. For example, the antidiabetic drug troglitazone (Rezulin) was removed from market after detecting its side effects like liver failure in millions of patients treated with this drug (Chojkier, 2005). Appropriate testing is therefore necessary to check out the safety and toxic concentration of a drug. Plants and its various parts contain various useful constituents and are rich in antioxidants, because of which it is considered safe against possible toxicity. Hence, it is widely used all over the world and used for the treatment of various diseases by general public and doctors as well (Oduola et al., 2007).

Although toxicity resulting by the use of herbs has been documented in literature, but the potential toxicity of herbs itself has not been acknowledged by the general public or by professional groups of traditional medicine practitioners (O'Hara et al., 1998).

Factors Influencing Toxicity:

Several plant materials are used for the treatment of various diseases and very little or no documentation is available on ingestion of plant materials. A very rare result is reported in the USA by accidental ingestion of poisonous plants (Krenzelok and Mrvos, 2011). Compounds like cyanogenic glycosides and lectins like phytohaemaglutinin are the main reason behind causing toxicity to the plants.

There are many plants which instead of being used as food may leads to toxicity at different stages of life by toxic parts of plants, unless it is processed. Some examples are seeds of Apple (Malus domestica Borkh.), causes toxicity if ingested in large amount because of the presence of cyanogenic glycosides. The nuts of Cerbera odollam Gaertn. (Suicide tree) having cerberin causes heart disease (Galliard et al., 2004). The presence of protoanemonin (Oslon, 2004) or ranunculin (Spoerke and Smolinske, 1990) in Helleborus niger L. (Christmas rose) leads to ulceration, gastroenteritis, hematemesis and burning of eyes, mouth and throat (Oslon, 2004).
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There are some other poisonous plants like Castor bean and *Ricinus communis* L., the seeds of which are very toxic and can cause nausea, cramps, vomiting, bleeding which leads to failure of metabolic system. They are documented under the world’s five most poisonous plants; even being toxic it is used by the natives of Africa as an additive and food (www.prlog.org).

Assessment of Toxicity:

Toxicology is the study of adverse effects of chemicals on living organisms and the extent upto which a substance can cause damage to an organism is called toxicity. Toxicity can affect the whole organisms or the substructure of the organisms, like cell (cytotoxicity) where a cell shows toxicity or organ (organotoxicity) where the toxin damages the whole of the organ. Toxicity is divided in three parts i.e. acute, sub-acute/sub-chronic or chronic.

Acute Toxicity

Acute toxicity leads to lethal effects following oral, dermal or inhalation exposure to a toxin. Acute exposures don’t last longer than a day and just a single dose of toxicant is effective enough to cause harm or to damage the body of the individual. The acute toxicity testing is used for gathering details on acute systemic toxicity in identification of hazards and management of risk related to the chemicals production and utilization.

The LD$_{50}$ value, (in acute toxicity test, the dose that causes death in a specific period to 50% of the treated animals), is the basic test used for chemicals toxicological classification. Both, mice and rats can be chosen for the study and for regulatory purposes both sexes must be used. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained (Walum, 1998). Efforts have also been made to develop *in vitro* systems; e.g., it has been suggested that acute systemic toxicity can be broken down into a number of biokinetic, cellular, and molecular elements, each of which can be identified and quantified in appropriate models. The various elements may then be used in different combinations to model large numbers of toxic trial to predict hazard and classify compounds.
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*Chronic Toxicity*

Sub-acute or sub-chronic is the period of toxicity which comes in between acute and chronic toxicity. Chronic toxicity refers to exposure of toxin for a prolonged period of time, which can be from months to years. Regular intake of alcohol can be taken as an example, where its ingestion for a long period of time causes organ toxicity. Toxicity mainly depends on the dose; even a very toxic substance like snake venom does not show toxic effects below a certain level of dose and on the other side a non-toxic agent like water used to show toxicity, if taken at a higher dose. A drug when given in a sufficient concentration or limit, it definitely causes some kind of toxicity in the body. The tolerance of the individual is also an important factor of toxicity. If there is any drug whose normal concentration causes toxicity in any individual, it may be due to genetic or immunologic cause and called as idiosyncratic drug toxicity.

**Target Organ Toxicity:**

The extent of toxicity varies from individual to individual and from organ to organ. The kidneys and livers are the most susceptible organs to toxicity because of their highly vascularised nature.

*Liver Toxicity*

The liver is the important organ of our body and its main function is metabolism, which regulate the body homeostasis. It also stores nutrients and other trace elements, helps in detoxification and breaking down of waste products, transportation of waste products to kidneys for excretion, and formation of bile juices for fast metabolism. The liver metabolises each and every kind of compounds, therefore the liver subjects to various diseases. This becomes a global concern, but medical treatments to this is not easily handled and also don’t have efficacy (Kim *et al.*, 2009). Liver can be called as a unique organ, as it is having many of host defence systems which protect it from various harmful compounds. One of which is regeneration (Mehendale, 2005) others include secretion of pro-inflammatory cytokines and acute phase proteins, gluconeogenesis, detoxification and clearance of endogenous mediators (Pastor *et al.*, 1995). There are so many plants which act as hepatoprotectants and used for various liver disorders, while others are reported to act as hepatotoxicants. *Glycosmis pentaphylla* (Retz.)DC.
protects mice hepatocytes membrane integrity (Nayak et al., 2011). Other like Artemisia macivera Hutch. & Calziel is reported to be hepatotoxic in nature when given in higher doses (Atawodi et al., 2011) and sometimes green tea also shows hepatotoxic side effect (Rohde et al., 2011).

**Kidney Toxicity**

The kidneys have plenty of functions and play a very important role in regulating the urinary system, serving homeostatic functions, like electrolytes regulation, acid-base balance maintenance and blood pressure regulation. It helps the body to act as natural filter by removing wastes, diverting it to urinary bladder. The wastes excreted are urea and ammonium in urine. Kidney helps in re-absorption of water, glucose and amino acids and hormones calcitriol, renin, and erythropoietin were produced by kidney. The kidneys play important role in filtering harmful substances from the blood. But sometimes exposure to chemical substances causes toxic effects to kidney, ureter, or bladder. Kidney injury occurs by exposures to various halogenated hydrocarbons, like carbon tetrachloride and trichloroethylene and heavy metals, cadmium and lead exposures causes kidney injury. Among which some leads to acute injury, while others cause a chronic change leading to renal failure or cancer (Toback, 1992).

Chloroform, for example, is nephrotoxic following metabolic activation via microsomal enzyme system. Chloroform is also hepatotoxic and again this involves cytochromes P-450-mediated activation, although in male mice the kidney is susceptible at doses that are not hepatotoxic. It is clear that the tissues of the kidney are often exposed to higher concentrations of potentially toxic compounds than most other tissues (Timbrel, 2009). Common disorders that affect the kidney include nephropathy, congenital hydronephrosis and obstruction of urinary tract among others. Several mechanisms and drugs are employed to treat these disorders.
OVERVIEW OF MYCOTOXIN:

*Fusarium* mycotoxins are mainly synthesized by *F. graminearum* and *F. culmorum*, and many other species of fungi. It includes more than 140 fungal metabolites and hence, considered the largest group of mycotoxins. Deoxynivalenol (DON), nivalenol and T-2 toxin were the most common *Fusarium* mycotoxins. Most of the animals and human health get affected and may leads to death, due to numerous fungi species produce it and its high toxicity. They are abundant in cereals and their products (Yazar and Omurtag, 2008). Among all common mycotoxins, *Fusarium graminearum* mainly produce mycotoxin DON (Kushiro, 2008). DON is polar organic compound, chemically belongs to family trichotheccenes and its chemical name is 12, 13-epoxy-3α, 7α, 15-trihydroxytrichothecc-9-en-8-on (Fig. 1.1). Structurally, it belongs to the type B trichotheccenes, The presence of 3 free hydroxy groups (-OH), in its molecule are related to its toxicity (Nagy et al., 2005).

After its consumption, it gives emetic effects, by running dopaminergic receptors in brain. Due to this reason it is also called as vomitoxin. DON is most commonly occurring grain and its products contaminant, representing more than 90% in food and feed. Also works as a marker for other mycotoxins occurrence (Sobrova et al., 2010).

DON exhibits a wide range of toxic activities, including gastro-intestinal toxicity, lymphoid toxicity, bone marrow toxicity, and cardiotoxicity as well (Forsell et al., 1987). But, the complete explorations of toxicity in terms of hepatotoxicity, nephrotoxicity, cytotoxicity, cellular apoptosis and genotoxicity have not been documented in totality. Again, the mechanisms by which this toxin damages the DNA and induces cellular toxicity are not well cited. Therefore, considering the above fact regarding the molecular mechanisms of DON toxicity leading to tissue necrosis, it was taken up to study the DNA damaging properties of DON in lymphocytes. Comet assay was also performed for evaluating DNA damage and bone marrow cells, while for evaluation of elastogenic and/or aneugenic activities caused by DON is studied by micronucleus assay. In spite of that, the occurrence of apoptosis was calculated using flow cytometry on the basis of morphological analysis of annexin and chromatin condensation.
AIM AND OBJECTIVES:

Keeping the above facts in mind the present study entitled “Effect of Meyna spinosa Roxb. ex Link and Oroxyllum indicum (L.) Kurz against mycotoxin induced toxicity” was taken up with the aim to assess the safety and efficacy of M. spinosa and O. indicum to control mycotoxin (DON) induced toxicity with the following objectives:

- To study the effect of Deoxynivalenol (DON) in toxicity induction on animal primary cell culture.
- To assess the safety profile of M. spinosa and O. indicum extracts.
- To study the antioxidant and hepatoprotective effect of the safe plant extract against DON-induced toxicity.