2. REVIEW OF LITERATURE

The rapid growth of human population coupled with the continued pressure on the environment has lead for tremendous technological progress to explore and exploit new areas and new resources to meet the needs of the modern man. Of late, the search of biologically active compounds from marine species has emerged as an important area for research. The foundation of searching for drugs from marine plants and animals has created a fantasy. Little is known about the history of the use of marine sources in traditional medicines, but it has been reported that marine algae have been used in Chinese folk medicine for more than two thousand years (Zeng et al., 1984). Documentation from around 40-90 A.D shows the use of marine invertebrates and fishes for the treatment of various diseases and ailments such as toothaches, ulcers and boils by the Greeks (Gunther, 1959). Research on Drug discovery and development from the ocean was initiated by the Bergmann and Fenney (1951) and it was expanded during the 1970s. Consequently, till now more than 10,000 compounds have been isolated from marine organisms (Proksch et al., 2002).

Marine Natural Products

Available information suggests that several medicines used to combat diseases of man and domestic animals have their origins from plants and animals. But for a long period of time, the pharmaceutical industries was using the terrestrial plants, animals and microbes obtain bioactive compounds that are used in Pharma and Biotech companies. These resources have now been over exploited and are not sufficient to meet the new challenge in human healthcare. This has
motivated researchers to explore and exploit the untapped marine plant and animal resources, which were considered inaccessible. Inspite, of this several chemical compounds have been isolated from marine species and their further chemical analysis proved as a potent source possessing various activities such as antimicrobial, anti-inflammatory, anti-diabetic, anticoagulant and anticancer. These biomolecules have successfully used against different ailments and diseases are hence termed as Marine Natural Products (MNPs). However, marine organisms such as sea weeds, mangroves, sponges, coelenterates, corals, molluse, echinoderms, sea snakes, fishes are increasingly attracting attention due to their production of structurally unique and pharmacologically active compounds.

Several chemical entities are under advanced preclinical testing and additional candidates for clinical development are emerging, including compounds hitting a specific target. Some important MNPs having convincing therapeutic properties are discussed hereunder.

Present cancer treatment is limited to surgical intervention that involves removal of infected body part and application of chemotherapeutic drugs and radiation therapy to destroy and inhibit the proliferation of cancer cells. None of these treatments actually cure cancer, but mostly only lengthen the lifespan of a patient. Again all these treatments, especially the chemotherapy and radiation therapy are associated with many life threatening side effects. Therefore pace has been made to search new compounds to combat cancer. It has been established that natural products are more effective in cancer treatments with minimum side effects.
Wesson (1996) isolated Keenamide A is a cytotoxic cyclic hexapeptide isolated from the marine mollusc *Pleurobranchus forskali*ii. It has exhibited significant activity against the P-388, A-549, MEL-20, and HT-29 tumor cell lines. Thus, it could also be a potential anticancer biomolecule of molluscan origin. Similarly, the Spanish Phamamar group has isolated the potent anti proliferative alkyl amino alcoholic compound namely Spisulosine ES-285 from the Arctic surf clam, *Spisula polynyma*. This bioactive compound has intriguing mechanisms of action which is now undergoing phase I clinical evaluation. The molecular target of this molluscan alkyl amino alcohol compound is Rho (GTP-bp) (Cudros *et al*, 2000). Lopez-Marcia, (2001) reported that Kahalalide F extracted from Hawaiian mollusca *Elysia rufescens* was found to possess good anticancer agent. It forms the largest and most active compound among the seven Peptides (six cyclic-A to F and one acyclic-G analogue peptides) isolated from *E. rufescens*. The first anti tumor compound, derived from tunicates was ET-743 originally identified in 1988 by Kenneth Rinehart’s team at the University of Illinois, Urbana Champaign and licensed to Pharma Mar in 1994 (Haefner, 2003). ET-743 also represents the first active agent against sarcomas developed in the past 25 years and has demonstrated a therapeutic potential in pretreated ovarian cancer (Takahashi, 2002).

Newman and Cragg, (2004) reviewed that there are significant numbers of interesting molecules derived from marine sources or have been synthesized as a result of knowledge gained from a prototypical compound, are either in or approaching phase II/III clinical trials in cancer, analgesia, allergy, and cognitive diseases. Aplidin is a cyclic desipeptide, induces cytotoxicity in a non-MDR/p53 dependent manner. The phase I program has investigated the feasibility of dose
dense schedules and have confirmed a positive therapeutic index in patients harbouring pretreated solid tumors and lymphoma (Jimeno et al., 2004). Bhakuni and Rawat (2005) gave expansive report about bioactive MNPs.

Kim and Wijesekara (2010) reported that marine organisms are rich source of structurally diverse bioactive compounds and a great deal of interest has been expressed regarding marine derived bioactive peptides because of their numerous health beneficial efforts. Moreover, many studies have reported that these peptides can be used as antihypertensive, antioxidant, anticoagulant and antimicrobial components in functional foods or nutraceuticals and pharmaceuticals due to their therapeutic potential in the treatment or prevention of diseases. Hussain (2011) addressed the anticancer drug discovery from an evolutionary perspective and present a series of case studies that demonstrate the natural compounds from marine sources as anticancer drugs.

Montaser and Luesch (2011) reviewed marine natural products as a new wave of drugs and stated that the largely unexplored marine world presumably harbors the most biodiversity may be the vastest resource to discover novel ‘validated’ structures with unique modes of action that cover biologically relevant chemical space. Liu (2012) reported the potential of marine natural products as new drugs on the horizon and new technologies and efficient collaborations between multidisciplinary scientists is essential to ensure the future success of marine natural products as new therapeutic chemical entities that can make a significant contribution to the cure of human disease.
Senthilkumar and Kim (2013) reported that numerous marine invertebrates based compounds have biological activities and also interfere with the pathogenesis of diseases. Isolated compounds from marine invertebrates have been shown to possess pharmacological activities and are helpful for the invention and discovery of bioactive compounds. On the basis of their bioactive properties, this review focuses on the potential use of marine invertebrate derived compounds on anti-inflammatory and some chronic diseases such as cardiovascular disease, osteoporosis, diabetes, HIV and cancer.

Mayer et al., 2013 presented a systematic review of the preclinical pharmacology of the Marine Natural Products literature during 2009–2011 with a similar format to previous reviews and which resulted from extensive searches of several databases, including Marinlit, PubMed, Current Contents® and Chemical abstracts. Previously, they have made reviews on marine pharmacology from 1998 to 2008. They discussed the pharmacology of structurally characterized compounds isolated from marine animals, algae, fungi and bacteria in a broad manner. This review illustrated that the marine pharmacology during 2009–2011, remained a global enterprise, with researchers from 35 countries, and the United States, contributing to the preclinical pharmacology of 262 marine compounds which are part of the preclinical pharmaceutical pipeline, which in 2013 consisted of 17 marine natural products, analogs or derivatives targeting a limited number of disease categories. Recently Martins et al. (2014) outlined the paths of marine natural products discovery and development, with a special focus on the compounds that successfully reached the market and particularly looking at the approaches tackled by the pharmaceutical and cosmetic companies that succeeded in marketing those products.
Marine Polysaccharides

Polysaccharides are complex sugar molecules made up of many blocks of simple sugar. The sugars are held together by the glycosidic bonds. There are several beneficial effects of polysaccharides such as supports healthy blood pressure, increases calcium absorption, lowers cholesterol and blood lipids, inhibit tumor growth, help to prevent certain cancers and neutralize the side effects of chemotherapy and supports healthy energy levels. It has the power to reawaken the body’s energy reserves, acts as an anti-inflammatory by supporting the body’s anti-inflammatory processes, helps balance immune function, combat autoimmune disease and encourages healthy immune function, promotes cardiovascular health and healthy livers. The meadow of marine polysaccharides is already large and escalating. Glycosaminoglycans (GAGs) or mucopolysaccharides are long unbranched polysaccharides consisting of repeating disaccharide unit. GAGs have high degrees of heterogeneity with regards to molecular mass, disaccharide construction and sulfation due to the fact that GAG synthesis, unlike proteins or nucleic acids, is not template driven and dynamically modulated by processing enzymes.

Usov (1997) reviewed the methods involved in the structural analysis of marine sulfated galactans including determination of monosaccharide constituents and depolymerisation by reductive hydrolysis, identification of disaccharides repeating units by NMR spectroscopy and sequence analysis by enzymatic degradation. Caceres et al. (2000) extracted and analyzed the biological activity of marine polysaccharides carrageenans from Chilean samples and suggested that the polysaccharide exhibited promising anti-herpetic activity.
Kolender and Matulewicz (2002) extracted the sulfated polysaccharides from the red seaweed and identified the presence of agar backbone with an unusual pattern of sulphation mainly at the 3- position of the α-1galactose units and the presence of xylose side chains at the position of the β-D galactose residues. Thakur and Thakur (2006) stated in their review that, deep sea hydrothermal vent microorganisms are producers of polysaccharides having remarkable chemical properties. Vijayabaskar and Somasundaran (2008) have isolated heparin from a marine clam *Marcia opima* and tested for its *in vitro* anticoagulant activity and compared against commercial heparins. Anumugam *et al.* (2009) analyzed GAGs of two marine bivalves *Tridacna maxima* and *Perna viridis* and both the species was found to contain variable amounts of GAGs in the form of heparin biomolecules as identified by metachromatic activity and agarose gel electrophoresis analysis. The structural characterization studies clearly demonstrated that heparin is the major GAGs constituents in the test animals. Saravanan and Shanmugam (2010) have studied low molecular weight sulphated GAGs from marine mollusc *Amussium pleuronectus* using chromatography. Roșoiu (2010) extracted the GAGs from small sea fish and studied the certain bioactive effects. These natural bioactive complexes demonstrate a significant inhibition action against hyaluronidase, collagenase and elastase, the enzymes implicated in the pathology of conjunctive, cartilaginous and bone tissues and favor the *in vitro* formation of collagen fibrils, these biochemical processes being dependent of the bioactive compound system concentration. Laurienzo (2010) declared that marine polysaccharides have immense role in pharmaceutical applications and its multidisciplinary approach are imperative in order to develop novel polymers useful as drugs or for healthcare in a larger sense.
Barahona et al. (2011) studied the antioxidant activity of polysaccharide Fucoidan through kinetic approach and concluded that activity varies with their sulphation pattern. Senni et al. (2011) stated that the therapeutic potential of natural bioactive compounds such as polysaccharides, especially Glycosaminoglycans, is now well documented, and this activity combined with natural biodiversity will allow the development of a new generation of therapeutics. Vijayabaskar and Somasundaran, again in 2012 isolated the heparin GAGs from another backwater clam Donax cuneatus and compared the anticoagulant activity with commercial heparins. Silva et al. (2012) reviewed on polymers and ceramics of biomedical interest and envisaged that biomedical field will be an area in which marine-derived materials will have a role of major relevance. Stonik and Fedorov (2014) reviewed the importance of marine low molecular weight natural products as potential cancer preventive compounds. Further declared that their synthetic derivatives are applicable in cancer therapy or cancer prevention too.

Pavao (2014) reviewed the importance of GAGs analogs from marine invertebrates and declared that these invertebrate glycans can be obtained in high concentrations from marine organisms that have been used as a food source for decades, and usually obtained from marine farms insufficient quantities to be used as starting material or new therapeutics.

**Hyaluronic acid**

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide composed of glucuronic acid and N-acetylglucosamine repeats via a α-1,4 linkage. HA has received much attention as a natural source of health and beauty. It is one of the chief components of the extra cellular matrix that contributes significantly to
cell proliferation and migration. Because of its high biocompatibility and its common presence hyaluronan is gaining popularity in cosmeceutical and biomedical fields. Since the application of the polysaccharide in the research field has been ramified, the interest in isolation of HA has been augmented to greater extents. Hadadian and Pirie (1948) detected the properties and preparation of HA from human umbilical cord. They also studied the effects of temperature and presence of different salts on viscosity of HA. The equal amounts of sulphated (chondroitin sulphate) and non-sulphated (HA) acid mucopolysaccharides were isolated from the extracts of the cortex and medulla of fresh kidneys from pigs, sheep and dogs was reported by Dicker et al. (1966). Mathieson and Pearce (1977) isolated minimal hyaluronate degraded from rat skin. It was isolated quantitatively from fresh rat skin by homogenisation in an edebo press, extraction with buffered saline, selecting elution from DEAE-cellulose and gel chromatography on sephadex G-200. Ignatova and Gurov (1989) reviewed the principles of extraction and purification of HA.

Laurent and Fraser (1992) studied nature, distribution, functions and turnover of HA. They also reviewed the anionic polysaccharides temperament, occurrence, role and its turnover period. Fraser et al. (1997) and Kogan et al. (2007) overviewed the occurrence and physiological properties of hyaluronic acid a natural biopolymer, as well as the recent advances in biotechnology production and preparation of the HA-based materials for medical application. Manasa et al. (2012) reviewed the properties, production, routes of injection, sources and application of HA.
Khue and Vo (2013) studied the effect of complex nutrients, temperature and salts on Hyaluronic acid production in *Streptococcus zooepidemicus* ATCC 43079 and Kotra *et al.* (2013) also studied the cost effective media optimization for the enhanced production of hyaluronic acid in *Streptococcus zooepidemicus* 3523-7.

Giji *et al.* (2013) has isolated and characterized the hyaluronic acid from the liver of marine stingray *Aetobatus narinari* and also studied the antipoliferation properties. Recently, they also made an overview on strategies involved in the isolation and characterization of HA from marine organisms (Giji and Arumugam, 2014).

**Analytical approach**

Swann (1969) of Harvard Medical School had performed studies on the structure of HA with the characterization of the product formed when HA is treated with ascorbic acid. Physical and chemical methods were employed for its characterization. It was concluded that HA has a molecular weight of 65kDa and its polysaccharide chain is not depolymerised by ascorbic acid. Nelly *et al.* (1973) had given a new method for quantitative determination of uronic acids with Metahydroxyl-diphenyl. It is simpler, quicker, more sensitive and more specific than other methods and it needs lesser amounts of fluid. It is recommended for determination of acid nucopolysaccharides in biological materials.

In 1999, Volpi performed the analysis of hyaluronic acid and chondroitin sulphate (unsaturated disaccharide) by HPLC and fluorometric detection with dansylhydrazine. This method was found to be superior to others and through this method the unsaturated disaccharides can be separated with good resolution. In the
year 1999, Bernice and Dale performed molecular weight determination of
hyaluronic acid by gel filtration chromatography coupled to matrix – assisted laser
desorption ionization mass spectrometry. The molecular mass determined using
MALDI - MS and the signal obtained from chromatography established a calibration
curve for other hyaluronic acid samples analyzed by gel filtration chromatography
system. The method was found to be a reliable method for molecular weight
characterization of polydisperse polysaccharides. Shinomiya et al. (2001) applied the
centrifugal precipitation chromatography (CPC) for the separation of fragments of
chondroitin sulphate (ChS) and hyaluronic acid. ChS and hyaluronic acid were
eluted into several peaks detected using UV-detector at 275 nm. The overall results
demonstrate that CPC may be a useful for separation of biopolymers such as
glycosaminoglycans which quantitatively produce precipitates in an organic
solvent mixture.

Armstrong et al. (2002) measured high molecular-weight hyaluronan in
solid tissue using agarose gel electrophoresis. The results showed that the tissues
evidenced an absence of hyaluronic acid with a molecular weight below 790,000
KDa. This procedure can be used to study changes in hyaluronan size in tissue
during inflammation and other pathological states. Volpi and Maccari (2003)
purified and characterised HA from Molluse bivalve Mytilus galloprovincialis this
was the first invertebrate species from which HA was extracted. The molecular
weight was found about 200 kDa. Cesaretti et al. (2003) standardized a sensitive
and reproducible 96 well assay of uronic acid permitting a rapid processing of a
number of samples with a very low consumption of reagents for the determination
of complex uronic acid bearing polyanions such as Hyaluronic acid, chondroitin
sulphate, derman sulphate and heparin.
Kakehi et al. (2003) had studied the separation and biological implications of hyaluronic acid. In order to examine its biological activities, accurate quantification of hyaluronic acid was determined using electrophoretic and chromatographic techniques.

Volpi et al. (2005) reported the detection of sub microgram quantities of hyaluronic acid and dermatan sulphate on agarose gel by sequential staining with toluidine blue and stains all. Lopes-Lima et al. (2005) isolated, purified and characterized the glycosaminoglycans in the fluids of the mollusc Anodonta cygnea for the better understanding of nacreous shell bio mineralization. The results showed that the existence of two different GAGs with similar contents in the two fluids which suggest that heparin sulphate like GAGs has a relevant role in the bio mineralization mechanisms acting as the calcium carbonate nucleator in the shell.

Volpi and Maccari (2006) devided electrophoretic approaches to analyse complex polysaccharides. The recent emergence of enhanced analytical tools leads a breakthrough in the field of glycomics. Agarose gel, HPCE, fluorophore-assisted carbohydrate electrophoresis (FACE) of GAGs and its derived oligosaccharides has been employed by them for the structural analysis and quantification. Electromigration procedures were developed to analyze and characterize the complex polysaccharides.

Zhang et al. (2007) have performed TLC for the analysis of glycosaminoglycan oligosaccharides through the use of polysaccharide lyases. This method allows for the rapid, semi-quantitative analysis of a wide variety of GAGs oligosaccharides. Analysis of hexosamine showed that the sample contained 96% of glucosamine, confirming the identification of HA. Hyaluronic acid-protamine
complex possessing glycosyl transferase activity was produced from the calf
vitreous by addition of protamine sulphate. The glycosyl transferase enzyme was
separated from protamine and treated with N-acetylimidazole, inhibition of the
enzyme activity was observed. Similarly it was inhibited by UTP and UDP
suggesting its role in the process of synthesis.

Kafedjuiski et al. (2007) synthesized and performed an in vitro evaluation
of thiolated hyaluronic acid for mucoadhesive drug delivery. Cumulative release
studies out of matrix tablets comprising hyaluronic acid-cysteine and the model
compounds FD demonstrated a sustained drug release for more than 12 hrs due to
insitu formation of intermolecular and intramolecular disulfide bonds in the
thiomer matrix. Pitarresi et al. (2007) had characterized new hydrogels based on
hyaluronic acid and α-polysapartylhydrazide. The in vitro degradation assays
performed in simulated physiological conditions as well as in the presence of
hyaluronic acid evidenced that HA-PAHY samples undergo a poor chemical and a
reduced enzymatic degradation unlike native hyaluronic acid.

Similarly, Panico et al. (2007) studied the effect of hyaluronic acid and
polysaccharides from Opuntia ficus indica (L.) cladodes on the metabolism of
human chondrocyte cultures. The end data showed the protective effects of
Opuntia ficus indica (L.) cladodes in cartilage alteration, which appears greater
than that elicited by hyaluronic acid commonly employed as visco supplementation
in the treatment of joint diseases.

Jiebing et al. (2007) had proposed an improved methodology for the
quantification of uronic acid, either by a calorimetric determination after treatment
with concentrated sulphuric acid and carbazole or by gas chromatography after methanolsysis and subsequent acetylation in xylans and other polysaccharides. In this work, the fundamental chemistry involved in the two methods has been evaluated and some modifications have been suggested to increase the accuracy for complete quantification of all individual types of uronic acid. Price et al. (2007) had clinically evidenced HA. It is a naturally occurring biopolymer whose molecular structure is highly conserved between mammalian species. They searched the literature for the purpose of a systemic review on wound healing and cosmetic practice. It was summarized that HA provided a resume of the current technologies available in fields such as skin regeneration, wound healing and cosmetic surgery.

Zhou et al. (2008) studied the effect of hyaluronic acid on 1L-1β-induced chondrocyte apoptosis in a rat model of osteoarthritis. Their study showed that hyaluronic acid could suppress chondrocyte apoptosis in 1L-1β-induced osteoarthritis mode. The suppression of inflammatory cytokine activity within the joint might be an important mechanism of the clinical action of intraarticular injection of hyaluronic acid in the treatment of OA. Fangnola et al. (2009), hyaluronic acid in hydrophilic contact lenses: spectroscopic investigation of the content and release in solution - spectroscopic methods for the determination of the content of hyaluronic acid in solution.

Akdamar et al. (2009) developed a method to separate HA from cell culture. The Glucuronic acid-imprinted poly microbeads have been synthesized by suspension polymerization using the metal chelating properties of Glucoronic acid
and N-acetyl glucosamine; which are the basic components of HA. It has also been stated that this method is simple, stable, durable, cheap and compatible with biological systems. The micro beads can be reused without loss in adsorption capacity several times.

Recently, Giji and Anmugam (2014) described the overview on strategies involved in the extraction with several methods as enzyme digestion, organic solvent, sodium acetate precipitation and microbial production. Beside this, characterization of HA by different analytical techniques from marine organisms also described.

**Antioxidant activity**

Foshi *et al.* (1990) and Trabucchi *et al.* (2002) both examined whether the *in vivo* studies of Hyaluronic acid and low molecular weight Hyaluronic acid would prevent oxygen free radical damage to granulation tissue during wound healing respectively.

Campo *et al.* (2006) reviewed the antioxidant and beneficial effect of Chondroitin Sulphate. Mandal *et al.* (2009) reviewed that antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. This review presents information about the antioxidant/antiradicals and their role in our body and also their presence in spices and herbs.

Krasinski *et al.* (2009) stated that the antioxidant properties of HA are related to both its molecular weight and its concentration. likewise, from squid eyes Dai *et al.* (2012) isolated two hyaluronic acid fractions from srird eyes (HA-1 and HA-2) with different relative molecular mass and determined for their
moisture retention capacity and antioxidant properties such as ferric ion reducing power and scavenging activity against DPPH and hydroxyl radicals. Zhao-hui et al. (2011) also studied the physico-chemical properties such as solution pH, viscosity, color and infrared spectroscopy and UV spectra of 60 Co-γ irradiation treated HA with sodium chloride solution was used to explore the effect on its. Moreover, the irradiated hyaluronic acid exhibited a gradual increase trend of scavenging capability on DPPH free radicals in a dose-dependent manner.

**Anti-proliferation activity**

Cancer results when clones of mutated cells survive and proliferate inappropriately disrupting this equilibrium. Cancer cells are characterized by accelerated proliferative capacity and resistance to apoptosis. Cell proliferation can be stimulated by physiologic and pathologic conditions. The proliferation of endometrial cells under estrogen stimulation during the menstrual cycle and the thyroid-stimulating hormone-mediated replication of cells of the thyroid that enlarges the gland during pregnancy are examples of physiologic proliferation. Many pathologic conditions such as injury, cell death, and mechanical alterations of tissues also stimulate cell proliferation.

The use of Hyaluronic Acid (HA) in the biomedical field has been the subject of interest of many researchers over the years. Many reviews are available on the use of HA for tumor targeting, drug delivery and tissue engineering. Chemical modifications of HA have also been extensively reviewed, namely on the conjugation with cytotoxic drugs, and also the chemical grafting of hydrophobic molecules to obtain amphiphilic micelles and stimuli responsive materials (Pedrosa and Gama, 2014).
Hyaluronic acid is a negatively charged polysaccharide that is found throughout the human body, as it is part of the extracellular matrix, both at the cell surface and inside the cells. It performs several functions in the body. It serves as scaffolding for other molecules, aiding in the structural integrity around the cells. It also interacts directly with cell surface receptors, CD44 and RHAMM, and through them signals is given that affect intracellular activities.

The fact that CD44 and RHAMM receptors are expressed in greater numbers on cancer cells offers an avenue of transporting and targeting anti-cancer agents to tumors, metastatic lesions and lymphatic tissues. Although Dr. Falk may have been one of the first doctors to recognize and utilize this capacity of HA, this function of HA is being utilized more and more today.

This important receptor, CD44, comes in several forms, a short form that is found on normal cells and longer variations that occur on cancer cells. The CD44 receptor is situated within the phospholipid membranes of the cells, with a part of it extending into the interior cell cytoplasm. There it interacts with the intracellular machinery involved in cell movement, which can lead to cancer cells breaking off and creating metastases in other areas of the body. Experimental research with the longer receptor form of CD44 demonstrated that when it was inserted into benign tumor cells, they became aggressive, metastasizing cancer cells. The enzyme in the body that breaks down hyaluronic acid is hyaluronidase. This enzyme also reduces the long form of CD44 into the short form, which is the normal and immobile form. Research with mice given human breast cancer demonstrated significant tumor shrinkage upon IV treatment with the hyaluronidase enzyme.
Later Mesa et al. (2002) studied anti-proliferative effect of topic Hyaluronic acid gel in gingival biopsies of patients with periodontal disease. Coradini and Perbellini (2004) reported Hyaluronan as a suitable carrier for a histone deacetylase inhibitor in the treatment of human solid tumors. High molecular weight high concentration HA inhibits U937 macrophage proliferation. This was demonstrated by Sheehan et al. (2004) considering the three factors that it affects; proliferation, apoptosis, and cell death. It was concluded that HA does not entirely arrests cell division but significantly slows it down and induces apoptosis. It was reported that HA has anti-inflammatory effects on the macrophage supported by the findings. HA negatively influences U937 cell proliferation while inducing apoptosis and that the dual anti-inflammatory mechanisms would be important for restoration of homeostasis and tissue function. Necas et al. (2008) made a review on Hyaluronic Acid, discussing its physicochemical and structural properties, mechanism of action, pharmacokinetics, toxicity and efficacy & its application both in vitro and in vivo. Gurski et al. (2009) studied the Hyaluronic acid-based hydrogels as 3D matrices for in vitro evaluation of chemotherapeutic drugs using poorly adherent prostate cancer cells. Alaniz et al. (2009) studied the effect of low molecular hyaluronan in inhibiting colorectal carcinoma growth by decreasing tumor cell proliferation and stimulating immune response. El-Safory and Lee (2010) described the cytotoxic and antioxidant effects of unsaturated hyaluronic acid oligomers. Karbownik and Nowak (2013) also described Hyaluronan towards novel anti-cancer therapeutics. Recently, Saturnino et al. (2014) studied the cell viability assay of Acetylated hyaluronic Acid.
Hepatotoxicity

Hepatotoxicity implies chemical determined liver injury. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury (Scott et al., 2003), among which drugs account for 20-40% of all instances of fulminant hepatic failure.

Previously, several works on hepatotoxicity was carried out with sulphated polysaccharides. Josephine et al. (2008) reported the role of sulphated polysaccharides from Sargassum wightii in Cyclosporine A (CsA) induced oxidative liver injury in rats. The effect of sulphated polysaccharides on CsA-induced hepatotoxicity was studied in adult male albino rats of wistar strain. The study resulted that sulphated polysaccharides can act therapeutically against CsA-induced hepatotoxicity. Xu et al. (2008) analyzed a water-soluble polysaccharide MP-I isolated from Mytilus coruscus. MP-I was subjected to investigate the protective effect on carbon tetrachloride (CCl4) induced liver damage in male Kunming mice. The results suggest that the possible mechanism is due to its antioxidant activity of MP-I. Karthikeyan et al. (2010) reported hepatoprotective activity of brown alga Padina boergesenii against CCl4 induced oxidative damage in Wistar rats. The protective role of brown alga Padina boergesenii showed potential activity through its antioxidant sparing action against CCl4 induced free radical damage. Zhang et al. (2011) reported the anti-cancer effects of polysaccharide and phycocyanin from Porphyra yezoensis. Ross et al. (2012) studied the hepatoprotective effects of aqueous sulfated polysaccharide extract from Sargassum siliquosum J.G. Agardh on Paracetamol-Induced Oxidative Liver Toxicity and antioxidant properties. Mayakrishnan et al. (2013) made a review on Cardioprotective activity of polysaccharides derived from marine algae. Mutawie
and Naggar (2013) studied the protective effect of *Sargassum crassifolia* against Nimbecidine–induced hepatotoxicity and nephrotoxicity in Wistar rats. Treatment with *Sargassum crassifolia* improved the pathological alternations in the liver and kidney which indicates the protective role of *Sargassum crassifolia* in modulating the hepatic and renal lesions.

However, Hyaluronic acid also showed effective results in hepatic injury studies. Williams *et al.* (2003) studied on Hyaluronic acid and endothelial damage due to paracetamol-induced Hepatotoxicity and reported damage to hepatic sinusoidal endothelial cells assessed by serum HA was greater in non-survivors than survivors. Kim *et al.* (2008) reported effect of gamma irradiated hyaluronic acid on acetaminophen induced acute hepatotoxicity. Hwang *et al.* (2008) isolated a glycoprotein from the brown alga *Hizikia fusiformis* and examined whether it could protect against acetaminophen (APAP)-induced liver injury *in vivo* and *in vitro*. Analysis of the effects of *Hizikia fusiformis* on APAP toxicity in rats revealed that the serum glutamic pyruvic transaminase level was restored to the control level and glutathione level was also increased by co-treatment with *Hizikia fusiformis* and APAP. These results indicate that *Hizikia fusiformis* may inhibit APAP induced liver injury in Sprague-Dawley rats and found that ERK activation was involved in the protective effect of *Hizikia fusiformis* against APAP-induced cell death. Therefore, propose that MAPK signaling is involved in the protective effect of *Hizikia fusiformis* against APAP-induced liver injury. De Avila *et al.* (2010) studied on Hyaluronic acid in the evaluation of liver fibrosis in patients with hepatitis C on haemodialysis and concluded HA as an accurate noninvasive marker in predicting significant fibrosis in patients with hepatitis C on haemodialysis.
Firdous *et al.* (2011) studied the hepato-protective potential of carotinoid *meso-zexanthin* against paracetamol, CCl₄ and ethanol induced toxicity. Histopathological studies of live tissue showed the hepatic potential of *meso-zexanthin*. Pandit *et al.* (2012) reviewed on drug induced Hepatotoxicity. Sharma *et al.* (2012) studied on hepatoprotective activity of *Adina cordifolia* against ethanol induce hepatotoxicity in rats. The effects of two different solvent extracts of *Adina cordifolia* were comparable with standard drug silymarin. The results of aqueous extract has shown more promising effect as compared to ethanol extracts of leaves of *Adina cordifolia*. Bannuru *et al.* (2014) reviewed on relative efficacy of Hyaluronic acid in comparison with NSAIDs for knee osteoarthritis. Njoku (2014) reviewed the Drug-Induced Hepatotoxicity on metabolic, genetic and immunological basis.