10. SUMMARY AND CONCLUSION

Marine species offer a great diversity of polysaccharides showing interesting biological properties mimicking those described for the mammalian Glycosaminoglycans (GAGs). In peculiar, Hyaluronic acid is a subset of the GAGs with lack of sulphate group has been an attractive subject because of its manifold emphasize in therapeutics. It is very palpable that HA is going to decree the pharmaceutical and cosmetic industry in near years. On bearing this in mind, the present investigation has been made to isolate the HA from marine bivalves and also studying their biological activities such as antioxidant, antiproliferation and hepatoprotective activities.

The GAGs was isolated from two bivalve species such as *M. casta* and *A. pleuronectus*. The crude GAGs mixture was purified using an anion exchange column for the separation of HA. After purification, the presence of HA was determined by evaluating their uronic acid content and other spectroscopical methods. The fractions exhibited three distinct absorbance peaks (1,2,3). The revealed three different peaks were pooled separately and used for further confirmation of HA. The pooled fractions of both the samples were analyzed in Agarose gel electrophoresis, showed the migration of unique, strong blue bands as typical to HA for peaks 1 and 2. The gel when stained with Stains-all no metachromasia was observed, whereas when stained with toluidine blue, single band showed metachromasia that corresponding to the 3rd peak which is responsible for sulfated groups. The net yield of *M. casta* and *A. pleuronectus* HA was estimated as 6.8 mg/gm and 4.02 mg/gm respectively. The HA of *M. casta*,
A. pleuronectus as well as the Human umbilical cord (standard) was detected at the retention time of 1.6 min in HPLC and the purity was also estimated as 96% and 93% for both isolated HA.

In the case of physiochemical analysis, both the species showed significant differences in the isolated HA composition. The amount of uronic acid and n-acetyl glucosamine was estimated as 49.4%, 47.7% and 31.6%, 37.1% respectively in M. casta and A.pleuronectus. Whereas the carbon, hydrogen and nitrogen content in the M.casta and A.pleuronectus was estimated as 26.86% and 29.38%, 5.83% and 4.72% and 1.24% and 2.31% respectively. The optical rotation of both isolated HA showed the levoratatory.

The viscosity based molecular weight on Ostwald Viscometer of both the isolated HA was calculated as 268 kDa and 373 kDa respectively. In order to confirm the presence of HA, the dye binding assay was investigated through UVspectroscopy. Hence, the free dye alone showed the spectrum of absorbance at 520nm, whereas the isolated HA of both the species showed the spectrum at 640nm. Besides, isolated HA was digested with hyaluronidase and the patterns of hexasaccharides and tetrasaccharides was identified by HPTLC at Rf value of 0.2 and 0.3 respectively.

In the case of structural analysis by FTIR, the revealed spectrum of isolated HA of M.casta and A.pleuronectus have the same numbers of major peaks between 3432.79cm⁻¹ and 2922.15cm⁻¹; 3437.15cm⁻¹ and 2929.87 cm⁻¹ respectively as similar to the standard HA. As per the literature, the above mentioned peaks region were corresponding to the hydrogen bonded O-H stretching and N-H stretching vibration of the N-acetyl side chain; methyl C-H stretch responsible for glucuronic
acid. In addition, the peaks viz., 1629.21 and 1405.95 cm\(^{-1}\) assigned to amide I group of C=O carboxyl and aromatic primary amine C-N stretching respectively. The primary alcohol C-O-C, C-O and C-O-H stretches was found at 1020.569 cm\(^{-1}\). Disulfides (C-S Stretch) were responsible by the 669.07 cm\(^{-1}\) stretch. Likewise, the presence of glycosidic linkages were also confirmed by the H1NMR studies. The glucuronic acid (GlcA) regions was found in 3.1- 3.9 ppm and 2.1ppm responsible for N-Acetyl glucosamine (GlcNac).

In the case of biological properties, the in vitro antioxidant activities of isolated HA of *M. casta* and *A. pleuronectus* against the DPPH free radical scavenging activities was found to be 61.8 % and 54.42% at the concentration of 1 mg/ml (P<0.05). The hydroxyl radicals scavenging activity of *M. casta* shows the maximum activity (75.30%) than *A. pleuronectus* (63.42%). The scavenging activity of *M.casta* and *A.pleuronectus* HA on ABTS radical was estimated as 74.19% and 71.35% respectively. Superoxide radical scavenging effect of *M. casta* and *A. pleuronectus* HA was determined as 76.89% and 73.03% at 1mg/ml concentration. The inhibitory rate of lipid peroxidation was found as 69.24% and 71.48% for *M. casta* and *A. pleuronectus*. In addition, the anti-proliferative activity of isolated HA was carried out on human ostosarcoma cell line (MG63). The HA of *M. casta* inhibited the proliferation of ostosarcoma cells at 23 µg/ml, whereas *A. pleuronectus* HA showed the inhibition at 42 µg/ml.

The hepatoprotective activity of isolated HA in APAP induced Wistar rat was evaluated by various biochemical parameters such as lipid peroxidation (TBARS and LOOH), enzymatic antioxidants (SOD, CAT andGPx), non-enzymatic antioxidant (GSH) and liver markers (AST and ALT). Hence, the levels of TBARS

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**SUMMARY AND CONCLUSION**
and LOOH in serum and liver tissue significantly increased in APAP induced group when compared to the control group. The levels was decreased significantly in animals treated with HA along with APAP group and HA alone group, when compared to APAP administered group. There was a significant increase in the activities of GSH, SOD, CAT and GPx proving the effect of HA along with APAP on enzymatic and non-enzymatic antioxidant activities. In APAP treated rats, the level of ALT and AST were increased, then the level of ALT and AST in APAP along with HA treated rats and they were decreased as compared to inducer group.

The levels of serum and tissue cholesterol, triglycerides and FFA were significantly increased in APAP treated rats, when compared to the control group. There was a marked decrease in the levels of cholesterol, triglycerides and FFA in group HA treated rats when compared to APAP administered group. Whereas, the levels of HDL cholesterol in serum and tissue were significantly decreased in APAP induced group, when compared to the control group. Treatment with HA along with APAP increased the levels of HDL cholesterol compared to APAP treated rats. Apart from the biochemical estimation, histopathological examination of liver of control and experimental groups were analyzed. The sections from the liver tissue of control group resembled as such of normal liver, portal areas were clear, the hepatic lobular architecture was also normal, whereas the sections of APAP treated liver revealed the damaged hepatocytes, the surface of liver was unsmooth, lobular structure was damaged. HA alone treated rats showed normal architecture of hepatocytes surrounding the central vein. The rats treated with HA with APAP showed normal hepatocytes with normal architecture.
In conclusion, the present study provides the conquer information about the isolation, purification and characterization of HA from marine sources. The biological activity of isolated HA shows the prominent antioxidant activity that could be considered as a natural antioxidant which is also exhibiting potent anti-proliferative effect on cancer cell line. The in vivo studies of isolated HA evidenced competent the heptoprotective activity against the APAP induced Wistar rat. Thus, laying a concrete for the future therapeutic development of isolated HA in field of pharmacology. On intact, the study clearly depicts the advantage of isolated HA of *M. casta* and *A. pleuronectus* could serve as an alternative source to meet the demand for commercial value.