10.1. SUMMARY AND CONCLUSION

Isolation of bioactive compounds is now turning toward potentially more selective ways in disease treatments, especially when concerning cancer. Sphingolipids (SLs) are ubiquitous constituents of eukaryotic cellular membranes that are involved in cell growth, proliferation, differentiation and apoptosis. These sphingolipid-regulated processes are crucial in cancer development and progression, and influence efficacy of anti-cancer therapeutics. Keeping this in mind, the primary objective of the present work has been designed to find the anticancer activity of the *D. dehaani* crab hemolymph in *in vivo* and *in vitro* with the special reference of the anti-cancer compound sphingolipids isolation and characterization.

10.2. IN VITRO ANTI-CANCER ACTIVITY

This present study was aimed to evaluate the anti-cancer properties of the brachyuran crab *D. dehaani* hemolymph against several human cell lines viz., HepG2, HT-29, Rhabdomyosarcoma, and A549. The effect of hemolymph on cell proliferation was determined by MTT assay. The cytotoxicity of the crab *D. dehaani* against vero cells was observed at 200 µg/mL. In the HepG2 Cell Line the half inhibitory concentration (IC$_{50}$) was detected at 75 µg/mL. The HT-29 showed half inhibitory concentration (IC$_{50}$) at 95 µg/mL. The hemolymph that produced half inhibitory concentration at 75 µg/mL on Rhabdomyosarcoma cell lines. The hemolymph that produced the maximal half inhibitory concentration (IC$_{50}$) at 75 µg/mL on A549 cell lines. Among the tested cell line HepG2 showed very good results. Hence it has been further investigated by DNA fragmentation studies. In a combined report anti-cancer value of the crab in cancer cures is noteworthy which is confined to HepG2 cell lines than the rest.
10.3. *IN VIVO* ANTI-CANCER ACTIVITY

To confirm the *in vivo* anti-cancer activity, the hemolymph was tested against NDEA in rat model. The hemolymph treated group showed a significant decrease in the number of nodules and nodule size when compared with NDEA treated group animals. NDEA treatment increased the relative liver weight to body weight compared to the control group. The body weights were significantly decreased in NDEA-treated animals as compared with control. Treatment of hemolymph to NDEA-treated rats significantly improved the body weight as compared to NDEA-treated animals. The histopathological examinations of the current analysis shows the normal architecture and cells cytoplasm of hepatic cells with granulated cytoplasm, central vein, small uniform nuclei and nucleolus. Hemolymph treated animals showed normal architecture illustrating the non-toxic nature of hemolymph. NDEA alone showed loss of architecture and tumor cells which were smaller than normal cells with granular cytoplasm and large hyperchromatic nuclei, whereas animals pretreated with hemolymph showed few neoplastically transformed cells and hepatocytes maintaining near normal architecture. Overwhelmingly, the histopathological analyses were revealed that the hemolymph of *D. dehaani* would be a great source for potent anti-cancer compounds.

10.4. ANTIOXIDANT ASSAY

The present study provides evidences for the development of HCC in NDEA treated animals. In biochemical assay the phase I and phase II assay revealed increased activity of cytochrome P450 and decreased activity of GST observed. Our results provide evidences that hemolymph could block initiation of NDEA-induced hepatocarcinogenesis. Generally liver damage induced by NDEA reflects instability of liver cell metabolism and membrane instability subsequently caused distinctive changes in the
serum enzyme activities. Administration of hemolymph reduced NDEA induced hepatocarcinogenesis, as shown by the reverted activities of AST, ALT, ALP, LDH, and decreased the concentration of bilirubin and α-fetoprotein. From the above revealed results pre and post-treatment of hemolymph effectively suppressed the NDEA-initiated hepatocarcinoma and alleviating lipid peroxidation through scavenging free radicals and enhancing antioxidant status and reverting liver marker enzymes. Hence we can conclude that this marine crab could be useful in the prevention of HCC.

10.5. CHARACTERIZATION OF ANTI-CANCER SPINGOLIPIDS

The active fraction of the purified hemolymph was subjected to NMR and ESI-MS/MS analysis. The proton NMR spectrum of sphingolipid fraction exhibited signals at δ 5.36 and 5.35 for the unsaturation and 6.43, 4.14, 3.96, 3.72, 3.48, 3.26 for methine and methylene protons next to oxygen/nitrogen. 13C NMR of the hemolymph was also in agreement for the presence of carbonyl group as well as unsaturation. The ESI-MS/MS spectrum exhibited intence signals for sodiated molecular ions [M+Na]+ of sphingomyelins (SM) identified as N-2O-Acetyl-12 pentadecenoyl sphingosine phosphorylcholine, N-9-eicosenoyl-sphinganine phosphocholine and the corresponding dehydro sphingomyelin, N-9-eicosenoyl- dehydro- sphinganine phosphocholine along with the ions at m/z 147, 184 characteristic of phosphocholine. Based on the CID-MS of ion fragmentation structure was assigned for the identified compounds.

10.6. CONCLUSIONS

In recent years the definition of “fundamental structural components of biological membranes” has become reductive for the
sphingolipids. In fact, this class of lipids constitutes a source of bioactive molecules which, together with the enzymes of the sphingolipid metabolism, are involved in signal transduction and in some fundamental cellular mechanisms as proliferation and its arrest, differentiation and apoptosis, embryogenesis and ageing regulation. Therefore inhibitors of sphingolipid metabolism enzymes could be used to regulate cell function in physiological and pathological conditions. Our studies proved that marine crab *D. dehaani* have the direct potential inhibitory action on liver cancer cells. Hence, it is anticipated that marine crab *D. dehaani* would be a useful pharmaceutical material to treat liver cancer.