SUMMARY & CONCLUSION
Survival and fitness of higher organisms depend on lower organisms. Even though higher organisms originated from unicellular organisms like blue green algae, O₂ in this universe is a gift of these organisms, excess of this gift became a threat and is proved to be a disaster and is responsible for several diseases. The vast biodiversity of nature might provide bioactive compounds that may be useful in the fight against chronic diseases. Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. So there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable. Many of the important and effective drugs used to treat chronic diseases are derived from plants and certain species of cyanobacteria. _Nostoc sphaeroides_ was found to have a significant antioxidant activity and it could inhibit lipid peroxidation. Lipid peroxidation might be involved in tumor promotion and progression of carcinogenesis. The extract significantly protected the croton oil induced lipid peroxidation in mouse.

Enormous research efforts are currently pursuing nutritional and botanical intervention of inflammatory processes. Chronic inflammation is regarded as an essential factor for the progression of the neoplastic process. Hence, the therapeutic intervention aimed at inhibiting inflammation, reducing angiogenesis and stimulating cell-mediated immune responses may have a major role in reducing the incidence of common cancers. The extracts of _N. sphaeroides_ were, thus, evaluated for acute and chronic anti-inflammatory activity. The ethanol, methanol and aqueous extracts of _N. sphaeroides_ showed significant protection against acute inflammation induced by the carrageenan and chronic inflammation induced by formalin in mouse paw edema model. The anti-inflammatory activity of ethanol extract was higher than that of methanol and water extract in the entire
inflammation model. The effective dose was found at 100mg/kg. The activity of aqueous extract was better than methanol extract.

Liver and kidney are the two important vital organs mostly affected by the drugs and xenobiotics. Oxidative stress in these organs might be one of the major etiological factors for the site specific carcinogenesis. Thus, compounds with higher antioxidant activity would be able to protect the vital organs from such chemically induced oxidative damage. The investigations were carried out to find out the protective effect of ethanol extract of *N. sphaeroides* against CCl₄ and paracetamol induced chronic and acute toxicity in liver. Treatment with the ethanol extract could restore the CCl₄ and paracetamol induced decline of the hepatic antioxidant status.

One of the hallmark activities of tumor promoters in animals is the ability of tumor promoters to recruit inflammatory cells to the application site and stimulate a respiratory burst in these cells. It is increasingly acknowledged that ROS and RNS play a key role in human cancer development. This hypothesis is supported by the increasing report on the role of antioxidants in preventing or delaying the onset of some cancers. Free radicals are involved in the initiation and promotion of multistage carcinogenesis. The croton oil promoted skin papilloma formation is mediated through the generation of free radicals from the inflammatory responds caused by the active component of croton oil i.e. 12-tetradecanoyl-13-O-phorbolacetate. The compounds with the anti-inflammatory and antioxidant activities can effectively block the tumor promotion induced by croton oil. Hence the effect of ethanol extracts of *N. sphaeroides* on the induction of skin papilloma promoted by croton oil was evaluated. Topical application of ethanol extract at a dose of 1mg and 5mg effectively prevented skin papilloma formation in the mouse skin. Though there is no
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difference in the average number of tumor/animals between the two doses of extracts tested, the 5mg applied group of animals showed marked delay of tumor development (prolonged tumor latency period) and decreased the percent of incidence.

In the present study we have located into several aspects of pharmacological activities of ethanol extract of *N. sphaeroides* which was not looked into earlier and give a rational explanation of these activities. Ethanol extract of *N. sphaeroides* was found to have significant activity on carcinogenesis. Carcinogenesis was induced by 7,12 dimethylbenz [a] anthracene (DMBA) as initiator and croton oil as promoter. Damage to DNA by oxygen free radicals is frequently postulated to cause mutations that cause initiation and progression of cancers. It was found that skin papilloma produced by DMBA and croton oil application was significantly inhibited by topical application of ethanol extract of *N. sphaeroides*. It was also observed that prior treatment of *N. sphaeroides* extract before DMBA application was also found to inhibit the papilloma formation indicating that *N. sphaeroides* could inhibit the initiation of carcinogen by DMBA as well as produce an inhibition of tumor cell promotion produced by croton oil.

The long term cytotoxicity and antiproliferative activity of the EEN was then assayed by a tetrazolium assay namely MTT, using a panel of 5 human cancer cell lines namely MCF 7, SW 480, HCT 116, HeLa and IMR 32. This was done in a dose and time dependent manner. The MEC showed maximum activity towards MCF7, HCT116 and IMR 32. The apoptogenic effect of EEN was estimated in cultured human cancer cell lines, by DAPI assay, mitosensor assay and caspase assay. DAPI assay was performed to visualize the morphological changes in cells treated with EEN. The EEN treated cells showed nuclear condensation or pyknosis
indicating apoptosis. Similarly mitosensor assay proved MMP and cytochrome c release in EEN treated cell lines, which is an indication of apoptosis. The EEN did increase the total cellular caspase activity in treated cell lines, which also showed that EEN could induce apoptosis. Hence the mechanism by which EEN impart its anti-tumor activity might be by the induction of apoptosis in cancer cells.

The acute and sub-acute toxicity of the extracts of *N. spheroids* were studied in rats. The results indicated that all the three extracts showed no acute or sub-acute toxicity. The liver function test, renal function test and hematological parameters after 30 days of drug administration showed that the tested doses (50 and 250mg/kg) were non-toxic to the animals. However, a slight increase in the activity of urea and ALP were noted in the 250mg/kg ethanol extract and methanol extract treated group of animals compared to the 50mg/kg treated and normal group of animals. This suggested that the therapeutic dose should be lesser than 250mg/kg especially for prolonged duration of treatment. The availability of drug is a critical factor for various diseases. Hence, the yield of the extract should be taken into consideration. The yield of the extracts were found to be in the decreasing order of aqueous > methanol > ethanol.