Scope and objective
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Neuroblastoma is a less diagnosed extracranial tumor that occurs in childhood; age between 1-3yrs. Neuroblastoma is mostly not treated in India, because even awareness about the disease is almost nil among health professionals, physicians and health departments. The reality is, disease dissemination among the child population is 1% of total Brain Tumors. For infants dying without any diagnosis, Neuroblastoma seems to be playing a major role. Neuroblastoma is a multi-organ cancer. It starts from adrenal gland of infants and metastasizes to kidney and other vital organs, soon spreads to spine and brain.

Neuroblastoma is intermediate of neuronal & endocrinal cancer. It occurs at the time of embryo formation and continues as a blast. There is no well defined treatment for Neuroblastoma. It is found through research that Neuroblastoma develops due to failure in molecular cellular regulation. It is reported that many oncogenes and the genes related to differentiation fail in regulation. The disease is caused purely due to aberrant gene expression, like an increase in expression of Survivin, TRK – A and Bcl – 2. Many other genes also play a role in Neuroblastoma in sub-levels, like MYCN, CDKs and RAS. Neuroblastoma can be treated effectively at gene-protein levels with respect to signal cascades. It is very necessary to find specific protein targets to control and treat Neuroblastoma. As a basic primary treatment some of the natural drug sources have been used in many therapies in alternative medicine like Ayurveda, Siddha, Unani & Homeopathy. All these natural drug sources have been reported to have efficacy in protein regulation. In this lineage of phytochemicals, alkaloids seem to have very good effect against cancers, especially Vinca alkaloids, of which some are commercially made and sold. Many other alkaloids also have been reported for anti-cancer activity in the pre-clinical level.
Scope & Objectives

Piper longum. L is well known raw drug in India and China for its bronchial diseases, dyspepsia worms, amoebiasis, carminative, digestive and antimicrobial properties. It cures cough, dyspnoea, leprosy, diabetes, piles, anaemia, cardiac and spleen disorders. Piperine also increases the bioavailability of other drugs. It is one of the important constituents in many Siddha and Ayurvedic formulations. Apart from being a drug, it is used as spice in many food preparations in Asian countries. It contains the phytochemicals like volatile oil, alkaloids; piperine, resin and piperlonguminine, a waxy alkaloid N-isobutyldeca-trans-2-trans-4-dienamide and a terpenoid substance. It also contains minerals like calcium, phosphorous and iron. Piperidine alkaloid piperine is the active principle in Piper longum. L.

In this present study the Piper longum. L fruit’s methanolic extract and piperine alkaloid are proposed for study in various methods and bring out the possibilities of preparing as drug for Neuroblastoma treatment in this preclinical study. The natural source of drugs are very well proven for anticancer activity, having low side effects, easily available, at low cost. These advantages motivate the research community to take up phytochemicals for anti-cancer activity and other studies. Alkaloids have a well proven effect towards cancer control. There is anticipation of Piperine playing a regulatory role in Neuroblastoma at the molecular level.

TRK – A, Bcl 2 and Survivin have been hypothesized as protein targets for Piperine in this present study for molecular analysis against Neuroblastoma, one of the least treated childhood cancers.

The prime objectives of the present investigation are hypothesized:

- To find the feasibility of the study with respect to TRK – A, Bcl2 and Survivin as direct/indirect targets for Piperine, using in-silico tools at the dry lab level.
➢ To obtain the drug, *Piper longum*.L seeds are to be soaked in methanol and crude extract prepared, and the same planned to be screened and validated for piperine and other phytochemicals.

➢ To analyze *in-vitro* drug activity against Neuro 2 A cell lines and viability assay using trypan blue under various concentrations of *Piper longum*.L extract and Piperine. MTT assay, to determine the anticancer activity by assessing anti-proliferation property of the *Piper longum*.L extract and Piperine.

➢ To investigate DNA damage by performing Comet assay in terms of apoptotic death of cancer cells by the effect of *Piper longum*.L extract and Piperine.

➢ To check cell morphology change in Neuro 2 A cell line incubated with *Piper longum*.L extract and Piperine using imaging techniques, light microscopy and SEM

➢ To analyse Molecular level changes in Neuro 2 A cell line by determining the drug activity using RT-PCR for gene expression of TRK-A, BCL-2 and Survivin. Western blotting to analyze proteins, TRK-A, BCL-2 and Survivin expression changes on treatment with N2A cell line.

➢ To evaluate the acute and chronic toxicity, if any, and to fix the optimum dosage of *Piper longum*.L for N2A induced Neuroblastoma in Swiss albino mice model, by assessing the histopathology, activities of pathophysiological marker Homovanillic acid.

➢ To investigate the anticancer effect of *Piper longum*.L and piperine by determining the physiological parameters such as survival time and body weight in control and experimental groups of mice.
➢ To determine the effect of *Piper longum.*L and Piperine for anticancer activity by assessing biochemical marker Homovanillic acid in urine sample of control and experimental groups of mice.

➢ To determine the effect of *Piper longum.*L and piperine for anticancer activity through biochemical profiling of glucose, protein, urea, uric acid, total cholesterol, free fatty acid, phospholipid, and triglyceride, in adrenal tissue of control and experimental groups of mice.

➢ To determine the effect of *Piper longum.*L and piperine for anticancer activity using hormone marker adrenaline in adrenal tissue of control and experimental groups of mice.

➢ To determine the effect of *Piper longum.*L and piperine for anticancer activity using metabolic and pathophysiological enzymes like creatine kinase, LDH, Glu 6 PDH, 5’ Nucleotidase, Na+/K+ ATPases, Ca2+ ATPases and Mg2+ ATPases and LPO in adrenal tissue of control and experimental groups of mice.

➢ To determine the effect of *Piper longum.*L and piperine for anticancer activity by enzymatic and non enzymatic antioxidants like SOD, CAT, GST, GPX and GSH in adrenal tissue of control and experimental groups of mice.

➢ To determine the changes in cancer tissue on negative regulation property of *Piper longum.*L piperine by performing histopathological and ultrastructural studies on the Neuroblastoma targeted tissue such as adrenal of control and experimental groups of mice.

➢ To investigate the anticancer activity of *Piper longum.*L and piperine by determining the immunohistological changes of protein TRK-A, Bcl-2 and survivin in the adrenal tissues of control and experimental groups of mice.
To investigate the anticancer activity of *Piper longum*L and piperine by RT-PCR analysis on targeted gene TRK-A, Bcl-2 and survivin in adrenal tissue of control and experimental groups of mice.

To investigate the anticancer activity of *Piper longum*L and piperine by Western blot analysis on targeted protein TRK-A, Bcl-2 and survivin in adrenal tissue of control and experimental groups of mice.

It is anticipated that the results in the present study would afford a scope for a thorough and extensive study that can lead to the identification of a novel drug source, which will be a launch pad in the treatment of Neuroblastoma. The results obtained were discussed in the light of relevant literature to substantiate the medicinal claims of *Piper longum*L and piperine.