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To Study the Radio and Chemomodulatory effect of *Nigella Sativa* Seed Extract in tumor bearing Mice with relevance to improvement of Cancer therapy.

FINAL REPORT

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Summary of the Project entitled

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Introduction:

Cancer globally ranks as the second-most leading cause of mortality. According to WHO, the estimated annual incidence of new cases would increase from the existing 10.1 million in 2000 to around 15.3 million in 2020, mainly due to apprehended increase in the number from 5.4 million to 9.3 million, in developing countries. Radiotherapy and chemotherapy, either alone or in combination, has been widely applied either to eliminate the cancer completely, or to extend the lifespan of patients by some years to a few decades longer.

Radiation and chemotherapy though effective, reliable and most commonly used modalities used for the treatment for human cancer, emerge with a high rate of failure and acute toxicities, that can affect a wide variety of organ systems and consequent dose limitation/ treatment failure, due to the very few molecular differences between cancerous and normal cells. Often the consequences of toxicity have profound effects on cancer patients with long-term remission that not only affect the therapy, but also the overall quality of life of the cancer patients. Further, to obtain better tumor control with higher doses of radiation, the normal tissues should be protected against radiation injury. Thus, there is a need to identify radio and chemo protecting compounds possessing a differential protective ability, so that effective and reliable therapeutic interventions are possible in patients undergoing radio and chemotherapy.

Several anti-oxidants have been tested and shown to protect normal tissues from the radio and chemotherapeutic toxicity by scavenging free radicals. Amongst, these antioxidant agents, amifostine has been approved for clinical use but is also associated with toxicity when administered intravenously such as hypocalcaemia, anxiety, and hypotension. Other cytoprotective agents such as sodium thiosulphate, mesna, and procainamide are not approved for wide clinical use due to lack of efficacy and/or non-selective cytoprotection of tumor tissues against radiation and chemotherapy induced toxicity. A search for more effective and less toxic radio and chemoprotectors is the need of the hour.

been reported to possess antioxidant properties, which protect normal cells from oxidative stress (Bestwick and Milne, 2001).On the other hand, they also exhibit prooxidant activity which contributes to their therapeutic actions. These compounds can behave as prooxidant and antioxidants depending on their concentration and cytosolic redox status (Girdhani et al., 2005). Several plant-derived anticancer clinical agents, such as taxol, vincristine, vinblastine, etc., are under intense investigation to explore their potential to enhance the sensitivity of tumor cells towards radiation-induced cytotoxicity.

Hence, a drug discovery effort to identify a compound/extract, which will provide preventative and/or therapeutic treatment, is essential. Over the last millennia herbal medicines have been used for the successful prevention and treatment of numerous diseases. Traditional medicinal plant products exert minimal side effects, hence, hold a great promise to their acceptability to the public, and relatively economical too.

One such herb is *Nigella Sativa* belonging to the family Ranunculaceae. In recent years, the seeds of NS have been extensively investigated both phytochemically and pharmacologically. Some of the reported pharmacological properties of NS include hypotensive, anti-nociceptive, uricosuric, choleretic, antifertility, anti-diabetic, anti-histaminic, anti-oxidant, anti-inflammatory, anti-microbial, anti-tumor and immunomodulatory etc. Though this plant has been extensively studied for various activities in normal animals, studies about its radioprotective and chemoprotective activity in tumor bearing animals have not yet been reported. This prompted us to evaluate radio and chemomodulatory effects of methanolic extract of *Nigella Sativa* (MNS).

The major objectives of this work include:

I. Studies in Nigella sativa

1. Preparation and evaluation of NS extracts viz macerated (MNS), Soxhlet (Sox) and fixed oil (FO) for various phytochemical constituents and selection of an extract possessing significant antioxidant activity for further studies.
2. Evaluating the chemoprotective effect of selected NS extract against cisplatin induced toxicities in tumor bearing mice.
3. Evaluating the radioprotective effect of selected NS extract in tumor bearing animals.

Studies on Chemomodulatory effects of MNS:

Chemotherapy too has been one of the major therapeutic modalities commonly used for the treatment for a variety of cancer patients. Though chemotherapeutic agents are a main stay in managing cancer, they trigger a myriad of untoward reactions in virtue of their lacked tumor specificity, and low safety margins. Besides, the development of resistance to chemotherapy adds another challenge to their utility in cancer management. Cisplatin is one of the most potent and commonly used anticancer drugs against solid tumors. Despite, its excellent anticancer activity, the clinical use of cisplatin is often limited by its undesirable side effects such as nausea, anorexia, nephrotoxicity, hepatotoxicity myelosuppression. Although the precise mechanism for the cisplatin-induced toxicity is not well understood, various data reported in literature indicate that cisplatin is preferentially taken up and accumulated in the liver and kidney cells, resulting in the enhanced production of reactive oxygen species (ROS) and decrease in antioxidant enzymes levels in the organs (Hae-Ran Park et al 2009). Therefore, in this study, we attempted to explore further, the role of MNS in cisplatin induced toxicity without interfering with its antitumor activity in tumor bearing mice.

The effect of MNS on cisplatin-induced nephrotoxicity, hepatotoxicity, haematological toxicity was done by evaluating the levels of blood urea nitrogen (BUN), creatinine (Cre) for nephrotoxicity, serum glutamate pyruvate transaminase (SGPT or alanine aminotransferase), serum glutamate oxaloacetate transaminase (SGOT or aspartate aminotransferase) for hepatotoxicity, and haemoglobin (Hb), White blood cell (WBC) and platelet counts (Pt) for haematologic toxicity both in normal as well as tumor bearing animals. Further, we also evaluated the levels of Lactate dehydrogenase (LDH) and Alkaline Phosphatase (ALP) in serum. Biochemical tumor markers are used to screen tumors for differential diagnosis, prognosis, monitoring the progress and also for assessing the response to therapy. These enzymes are unique as changes in their activities; reflect the effect on proliferation of cells. Further since, proliferating cells are known to possess a growth potential and metabolic turnover dramatically different from those of normal cells, the rise in their activity is shown to be in good correlation with the number of transformed cells in cancer conditions.

To summarise, MNS was able to ameliorate the toxicity associated with cisplatin treatment as well as augmenting its antitumor effect, thus indicative of a cytoprotective effect of MNS in tumor bearing animals. This would have beneficial implications for patients undergoing chemotherapy with Cisplatin.

taken on the day of sacrifice of the animals did not show a significant reduction in volume after combination treatment of radiation and MNS which could be attributed to the short duration of pretreatment with MNS and a single radiation dose. The dose of MNS (100 mg/kg) could also have contributed to the non-observance of reduction in tumor volume as well as the WBI given, which was done as we wanted to ascertain the radiation effect on normal tissues and had we given local radiation we would not have been able to quantify the damage of radiation to the normal tissues.