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REVIEW PAPER

Radiation Induced Gastrointestinal Damage and Protection: *Nigella Sativa* Seed Extract and Thymoquinone

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ABSTRACT

Ionising radiation therapy is a common treatment for different types of cancers. The side effects associated with radiation includes destruction of normal cells, especially the dividing cells. The cells in the gastrointestinal (GI) tract and bone marrow are the primary targets. The GI damage is reflected by early histological changes, functional alterations and symptoms of nausea, vomiting and diarrhea. This has been designated as the radiation syndrome. Many synthetic drugs have been used to treat GI disorders but a definite cure has not been discovered so far and these available medications also cause several side effects. The herbal extracts are being tested for long time as preventive food supplement/drug in this disease. The radio protective effects of *Nigella sativa* (black cumin, (Ranunculacea) is already reported but its mechanism of action is not well established. Here in this review this aspect has been explored with special reference to various *in vitro* and *in vivo* models.

Keyword: Nigella sativa; Thymoquinone; Radioprotector; Herbal; Radiation

1. INTRODUCTION

Radiation accidents and excess use as medical intervention are both linked to health hazards. Its manifestation can appear within a week and it is also dependent on the dose of radiation. Mainly the dividing cells such as the cells of hematopoietic and gastrointestinal systems are the primary targets. Radiation therapy plays an important role in the management of cancer[1]. The gastrointestinal system often shows clinically relevant lesions induced by ionising radiation[2]. Accompanying injury to the surrounding intestinal tissue may result in serious morbidity and occasional mortality. The radiation enterocolitis is a major clinical problem[3]. Cancer patients undergoing radiotherapy have suffered from adverse effects related to the formation of free radicals, which cause gastrointestinal injury that the mucosa of the gastrointestinal tract (GIT) undergoes continuous stress and in order for its functions to remain unimpaired, it must renew itself rapidly to replace lost cells. This fast turnover supported by marked mitotic activity makes the alimentary tract mucosa extremely radiosensitive [4]. The late radiation effects include pathogenesis of cancer, pulmonary fibrosis and chronic or progressive heart and kidney diseases. Although, these conditions are highly dangerous but its better management is possible on its early detection.

Earlier, the radiation dose is estimated in terms of changes in hematological, biochemical and cytogenetic parameters specially the C-reactive protein, amylase, and transforming growth factors, lymphocyte depletion kinetics, clinical observation, and the dicentric chromosome (DC) assay etc but now miRNA in the blood samples are being proposed to be a better and sensitive biomarker in this field. These miRNAs are highly stable and can be detected in serum, plasma and urine. They are small in size ranging 19–22 nucleotides and protected in exosomes. Its abundance is measured by PCR based methods, but it has limitation due to its lower concentration. Now, digital amplification-free quantification and comparison method is being used which enables its evaluation of relative abundance of individual miRNAs. They reported the dose dependent decline in miRNA-150 and miR-342-3p along with increase in miRNA-200b and miRNA-762 in serum[5].

2. RADIATION AND THE GASTROINTESTINAL TRACT

Exposure to different forms of ionising and non-ionising radiations is almost unavoidable in daily life, especially during radiotherapy. Radiation-induced cell changes may result in death of the organism, death of the cells, modulation of physiological activity, or induction mutations resulting to different cancers that have no features distinguishing them from those induced by other types of cell injury[6]. Abnormal cancer cells are more sensitive to radiation because they divide more quickly than normal cells, but the normal cells are also affected. However this damage is efficiently repaired by the normal cells and over the time, the abnormal cells die out and the tumor shrinks. Radiotherapy is one of the major mode of efficient treatment, especially in case early diagnosis. e.g.

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cancers of the skin, cervix, prostate, lungs, thyroid and brain), and may be the only treatment needed to cure the cancer[7]. Nevertheless, radiation therapy remains dose limited by the tolerance of surrounding normal tissues.

The gastrointestinal tract (GIT) not only is a major dose-limiting organ during abdominal, pelvic, and retroperitoneal radiation therapy[8] but is also one of organs for the outcome of accidental whole-body radiation exposure. An exposure to the dose of 5 Gy - 8 Gy, may result to death within few weeks. However low dose in the range of 2 Gy - 5 Gy, may result to delayed symptoms especially to the hematopoietic system, resulting immunosuppression and rapid manifestation of infections. The GI damage is another cause of death depending on the dose and severity of damage, including intestinal crypt cell death. Therefore, interventions that reduce gastro-intestinal radiation injury are urgently needed. Radiation enteritis is a challenging clinical problem in patients receiving ionising radiation[9]. Clinically, irradiation can result in several deleterious gastro-intestinal symptoms such as bleeding, anorexia, nausea, vomiting and diarrhea i.e. gastrointestinal radiation syndrome[10,11]. The high sensitivity of the GIT to ionising radiation (and other cytotoxic insults) is a dose limiting issue in some radio- and chemotherapy practices. The duodenum is the most radiosensitive region of the digestive tract, followed by jejunum, ileum, esophagus, stomach, colon, and rectum, in order of radiosensetivity[12,13].The mucosal cells are characterised by rapid proliferation rate and relatively high degree of radiosensetivity. In both animal models and patients, small intestinal and whole gut transit was markedly accelerated within hours after irradiation[14,15]. The acute injury to the intestinal mucosa is the major dose-limiting complication of the abdominal gamma-irradiation while the colon has relatively high radio tolerance, possibly due to the long turnover of its cells[16].

Ionising irradiation induces a series of events leading to more complex alterations in GI physiology and structure. The effect of ionising radiation on gastric function is reflected by evidence of early histological damage, functional alteration, and signs and symptoms of the radiation syndrome[17]. Moreover, ionising radiation has several effects on cellular components such as development of the radiation induced reversible cellular injury. This is also accompanied by a series of morphologic alterations in the ultra structure of both epithelial and stromal cells and components, marked changes in the nuclear shape (swelling, irregularity of the border, dilatation of the nuclear envelope), mitochondrial swelling, de-granulation and vesicularization of the endoplasmic reticulum, enlargement of the Golgi complex, redistribution and/or reorganisation of the cytoplasmic actin and cytokeratin filaments[18-23]. Acute radiation gastro-enteritis is a result of mitotic cell death in the gastrointestinal crypt, disruption of the epithelial barrier, and mucosal inflammation. Mucosal injury is described as destruction of crypt cell, decrease in villous height, and number, ulceration and necrosis of the intestinal epithelium[24,25]. Although the pathogenesis of radiation gastro-enteritis is not clear, it is presumed to be an inflammatory process in which various mediators such as pro-inflammatory cytokines, and reactive oxygen metabolites take place[26, 27].

3. RADIOPROTECTIVE NATURAL PRODUCT

Several synthetic and natural products have been claimed to radioprotective. One of the synthetic product named as amifostine (WR2721; 2-(3-aminopropyl) aminoethylphosphorothioate) has been successfully used as approved drug by FDA, but it is associated with severe side effects and has a very narrow therapeutic time window. Further another product namely granulocyte colony-stimulating factor (G-CSF), has recently been approved by the US FDA. However a number of medicinal plants have been screened for their radioprotective potential. They may not be so effective as the synthetic drugs but they have lesser or undetectable side effects. These plant extracts may be used as prophylactic measure for patients undergoing radiotherapy[28].

Plant extracts are associated with several pharmacological potentials. Some of them includes radioprotection, anti-inflammatory, anti-oxidant, anti-fungal, anti-bacterial, anti-helminthes, immunomodulation, etc. these potentials are attributed to the presence of variety os secondary metabolites, especially the poly-phenolic, compounds, flavones, tannins, isoflavones, etc. literature is full of such studies from extract of single plant and also from the extract isolated of cocktail of many plants[29].

These includes cruciferous vegetables (e.g. Cabbage, broccoli), green tea (polyphenols), *Spirulina platensis, Menthe arvensis* linn (mint), *Podophyllum hexandrum* Linn. Rubia cordifolia, *Ocimum sanctum* (Tulsi) *Syzygium cumini*, Skeels (jamun), *Panax ginseng* Linn. *Aspalathus linearis*, soy products, *Ginko biloba* Linn extract, *Nigella sativa* Linn, grape seed extract, triphala extract, shigoka extract, curcumin, chlorogenic acid, Quercetin, garlic, lycopene, methylxanthines, melatonin, ellagic acid etc[30].

The plant extracts like *Centella asiatica* and Hippophae rhamnoids- fruit have reduced the adverse effects of radiation in rats. They have shown reduction in weight loss and also enhanced the life span[31-32]. One study reported that alcoholic root extract of *Rubia cardifolia* provides significant protection against radiation induced lipid peroxidation, haemopoietic injury and genotoxicity [33]. Similarly extracts of *Phodophyllum, Emblica officinalis, Tinospora cordifolia* and Ginger have shown protection of radiation induced damage to GI and embryonic nervous system and micronuclei formation and oxidative stress in mice respectively[34-36].

The protective effects of many compounds against radiation-induced gastrointestinal injuries have been investigated[37]. Currently the efforts are being made to search novel radioprotective agents from plants. This could protect the organism from different kinds of radiohazards e.g. immuno-suppression, genetic abrasions, teratogenic effects. The efficient approach for this task could be to evaluate the established phytochemicals having anti-inflammatory, antioxidant, antimicrobial, immune-modulatory, free radicals (FRs) scavenging or anti-stress potentials. Although some of the synthetic antioxidants have been screened as radioprotector, but they are associated with several adverse effects[38]. The phytochemicals having antioxidant potentials are mainly poly-phenolic compounds, such as flavones, tannins, isoflavones etc are being screened on priority, so that they can be launched

either as drug or as food supplements. Some of them, directly scavenge the FRs, generated during radiation exposure and som induce the endogenous antioxidant enzymes e.g. SOD, catalase, Glutathione peroxidase, etc.

Nigella sativa: This plant (dicotyledonous, Ranunculaceae) is described in Ayurveda and also in traditional medicine of other countries including Chinese medicine. Its seeds(black cumin) is a component of Indian spices and used regularly in Indian kitchen. Its oil is attributed to treatment of several diseases e.g. asthma, obesity, inflammation, fever, influenza, skin diseases etc. experimental studies have indicated its anti-histaminic, hypocholesterolemic, antioxidant and anti-inflammatory potential, anti-convelsent, anti cancer and anti-microbial activities. Interestingly the polar fraction of these seeds, extracted by methanol, also have antioxidant and anti-inflammatory potential[39]. Recently its radioprotection role has also been reported[40]. It protects radiation induced intestinal damage, improves survival, body weight recovery and normalisation of intestinal villi after high dose abdominal irradiation[41]. These therapeutic properties are attributed to its prominent phytochemicals e.g thymoquinone, thymol, carvacrol, nigellicine and its derivatives, myristic acid, saturated and unsaturated fatty acids and various trace elements and minerals[42].

The *Nigella sativa* seed extract show multi-targeted action because of presence of different secondary metabolites in fairly high concentration. For its radioprotective activity it involves different signalling pathways simultaneously to achieve the goal of prevention of cell death due to radiation. These may include the prevention of the pathways involved in oxidative stress, inflammation, apoptosis and gastric acid production[43,44].

Whole body exposing to acute doses of gamma irradiation either (6 Gy), were found to exert an oxidative stress due to generation of ROS, which characterised by elevation in TBARS and nitrite contents and depletion in reduced glutathione, as well as elevation in MPO activity and enhancing the production of cytokines such as TNF-α[45], an effect which could have been due to ROS-mediated activation of the transcription factor NF-κB[46-47].

Pretreatment of NS seed extract and TQ protect the tissues from oxidative stress induced by irradiation through protection against elevation in TBARS. Furthermore *Nigella sativa* is a powerful antioxidant that is able to induce some antioxidant enzymes and eliminate oxygen free radicals known to damage the gastrointestinal mucosa[48].

It is reported polar methanolic extracts of NS seeds (NSM) significantly enhanced antioxidant properties of irradiated rats by reducing lipid peroxidation in small intestine and by increase the activity of SOD, catalase and decrease the MDA level in intestine and also induce endogenous antioxidant enzymes (SOD, catalase CAT and glutathione peroxidase GPx) when rats are treated with *Nigella sativa* and TQ. It has potential to directly scavenge the active free radicals from the system through electron donation and delocalisation in the electrons in the aromatic rings[49-50]. Several studies have reported its beneficial role against variety of cancer cell lines such as cervical, breast, bone, stomach, colon, prostate and lymphoma[51].

Pretreatment with NS seed extract and TQ protect against inflammatory reaction induced by irradiation through protection against TNF-α elevation and enhance PGE2 production by prevention of increased tissue MPO activity. This observed gastroprotective effect of NS seeds is mostly attributed to its phenolic components such as flavanoids and phenolic acids which were shown to exhibit cytoprotective properties[52].

In one report the anti inflammatory effect of TQ is evaluated by the level of the pro-inflammatory cytokine; TNF-α, in intestinal mucosa, as well as intestinal expression of NF-κB and COX-2. It is found that TQ down-regulates inflammatory markers as, TNF-α, NF-κB and COX-2, as well as inhibition of apoptosis[53]. Other study results showed that [54], total nitrite/ nitrate levels, as well as expression of iNOS, are the indicators of nitrosative stress. TQ treatment cause significant reduction of iNOS and protein kinase C (PKC) expression in intestinal cells, there by reducing their associated toxicity[55].

In previous results it was shown that, methanolic fraction of NS seed prevent irradiated induced loss of villi, shortening of villi height, and flattened crypts, with focal loss of intestinal epithelial cell lining collagen deposition. This is due to presence of TQ and several phenolic compounds[56]. A study based on albino rats showed that NS, TQ and its chief constituents show role in gastric ulcer prevention via decreasing gastric acid production/increase gastric mucosa and by increasing the bio-availability of arachidonic acid, the substrate for synthesis of prostaglandins in stomach[57]. On the contrary it inhibits the synthesis of leukotrienes, responsible for mucosla tissue injury and hypoxia[58]. Thus it alters the balance between these to eicosanoids towards its cytoprotective response.

In this review we hypothesize that the powerful antioxidant activity of NS seed extract and TQ prevents radiation-induced oxidative stress from initiating intestinal damage, which, in turn, prevent the activation of TNF-α/NF-κB/COX-2 inflammatory pathway, as well as the subsequent triggering of automated cell death; apoptosis[59].

The protective effect of TQ against radiation-induced toxicity raises the question whether it confers similar protection to cancer cells. However this paradoxical situation has been ruled out as several studies have shown its antitumor potential on variety of cell lines . Thus based It has shown to inhibit Akt phosphorylation, which leads to DNA damage and apoptosis in cancer cells. The same group has shown that it has synergistic activity cisplatin, suggesting its beneficial therapeutic response and action through different signaling pathways[60]. Another study also show production of free radicals and P38 phosphorylation. in breast cancer cell line, thus inducing proapoptotic potential[61].This is just opposite what we observe in normal cells, where it scavenges FRs. Thus it role in normal and cancer cells are different.

On the above mentioned findings, the gastroprotective activities of NS seed extract and TQ as well as their anti-inflammatory reactions and their ability to reduce oxidative stress indicates that, their possible preventive value in the inhibition of tissue damage involving free radical reactions. Thus, these drugs could have the potential to safely and effectively enhance the benefits of radiotherapy, by the

management of excessive gastrointestinal damage induced by radiation exposure. Further clinical investigations are required to assess the beneficial health effects of these drugs and hence support their use.

4. CONCLUSIONS

The main constituent of *Nigella sativa*; TQ, confers intestinal protection against radiation-induced mucositis. The mechanisms of protection include multi-targeted action through interfering the radiation-induced oxidative/nitrosative stress, inflammation, and apoptosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this paper.

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In the current study, he helped in designing of the manuscript and final corrections.