Abstract of the PhD thesis

Title of the Thesis: Role of potential antioxidants at lethal/sub-lethal doses of γ-radiation: Investigation under *in-vivo* and *ex-vivo* animal model systems

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Background: Exposure to whole-body radiation may results in multi-organ dysfunction leading to cause acute radiation syndrome (ARS)-includes haematopoietic, gastrointestinal and cerebrovascular sub-syndromes. Therefore, development of radioprotector must target these sub-syndromes. Radioprotectors are prophylactic agents that are given prior to radiation exposure to protect radiation-induced injuries, either by preventing the initial injuries, or reconstituting the original structure by repair, or by both.

Selection of Potential Antioxidants: In the present study we have screened sixteen antioxidants molecules using various standard parameters. Out of these, sesamol and melatonin has shown strong antioxidant potential. In this study, therefore, we have used sesamol and melatonin for further radioprotective evaluations under *in-vivo* and *ex-vivo* animal model.

Toxicity: The safety and toxicological studies are essentially required to identify the range of safe doses that could be used subsequently in pre-clinical model for investigating the therapeutic index. In this study, therefore, we have determined the safe dose of sesamol and melatonin by following internationally recognized "Acute Oral Toxicity method OECD 423". Both the sesamol and melatonin would place under GHS category 4 (>300-2000) molecule with LD_{50} cut-off value of 500 mg/kg (for sesamol) and 1000 mg/kg (for melatonin) in female C57BL/6 mice. However, we have used 100 mg/kg of sesamol ($1/5^{th}$ of LD_{50} dose) and melatonin ($1/10^{th}$ of LD_{50} dose) for the pre-clinical evaluations in animal model.

Survival: The survival study is considered as one of the gold standard endpoint evaluation for ARS. In this study, therefore, we have conducted survival study with different doses of sesamol and melatonin ranging from 50-150 mg/kg body weight against $LD_{50/30}$ dose of 7.5 Gy whole-body irradiation (WBI). Both the sesamol and melatonin provided 100 % survival against $LD_{50/30}$ dose of 7.5 Gy.

Haematopoietic Systems: The radiation-induced haematopoietic injury is mainly manifested by the loss of bone marrow haematopoietic progenitor stem cells (HPSCs). We have performed colony forming unit assay, cell cycle and comet assay to assess the radiation-induced (2-7.5 Gy) malfunction in HPSCs. Analysis of results depicted that both the sesamol and melatonin markedly protected the radiation-induced loss of functional HPSCs, cell cycle perturbation and DNA strand breaks in HPSCs, therefore, overcoming haematopoietic injury in irradiated mice.

Immune Systems: Spleen is considered as one of the important tissues for immunological studies and plays an important role in immune system, and hence immunomodulation. In the present study, therefore, we have

conducted a morphological examination of spleen, loss of T and B cells populations, apoptosis, DNA strand breaks and proliferation of splenocytes. Our findings revealed that both the sesamol and melatonin significantly protected hemorrhage, apoptosis, DNA strand breaks and enhanced recovery of T and B cells population and proliferation of splenocytes, thereby, played an important role in immunomodulation of irradiated mice.

Gastrointestinal System: The gastrointestine (jejunum) is considered one of the most vulnerable systems to radiation-induced injury, next to the hematopoietic system. In this study, therefore, we have performed histopathological examination, lipid peroxidation, DNA strand breaks and gut bacterial translocation assay to evaluate the radiation-induced gastrointestinal injuries. Pre-treatment of sesamol and melatonin remarkably protected the gastrointestine from radiation-induced injuries as evident by normal villi and crypt morphology and number, reduced level of lipid peroxidation, DNA strand breaks and gut bacterial translocation to the spleen, liver and kidney. Thus, these results confirm that both the sesamol and melatonin played important role to overcome radiation-induced gastrointestinal injuries.

Reproductive System: Exposure to ionizing radiation can cause reversible or permanent damages in male reproductive system. Testis is one of the most radiosensitive male reproductive organs, sensitive to radiation as low as 0.1 Gy, because of possessing highly proliferating spermatogonial cells. In this study, therefore, we have evaluated radiation-induced spermatogenic lineage and stage alterations, azoospermia, spermatogenic proliferations, lipid peroxidation, DNA strand breaks, sperm abnormalities, sperm motility and viability to assess the testicular malfunctions. As anticipated, both the sesamol and melatonin remarkably protected the spermatogenic lineage and stage alterations and azoospermia by inhibiting lipid peroxidation and DNA strand breaks and enhancing the proliferation of spermatogenic cells. This protection led to the reduced level of sperm abnormalities and enhanced level of sperm viability and motility. Thus, these findings suggest that both the sesamol and melatonin provided significant level of protection to spermatogenic cells against radiation-induced injuries, thereby, overcoming the testicular injuries.

Antioxidant System: Biological molecules are most susceptible to oxidative damages when antioxidant defence system is dominated by radiation-induced ROS. Assessment of total antioxidant capacity (TAC), therefore, is expected to provide imperative information related to radiation-induced oxidative damage and recovery of radiosensitive organs in rodents. Analysis of results indicated that both the sesamol and melatonin significantly enhanced the level of TAC in spleen, gastrointestine and testes. Thus, findings suggest that the increase of TAC could overcome the imbalance between pro-oxidant and antioxidants, and leads to reduced ROS-mediated cellular injury in WBI mice.

Mechanism of Actions: Elucidation of the molecular mechanism of radioprotective drug in more than one organ is crucial and plays a vital role in the new drug approval process as per the United State FDA`s "Animal Efficacy Rule". To prove this hypothesis, we have measured the expression pattern of ATM, p53, p21, Bax, Bcl-x, cytochrome C, caspases-3 and caspases-9 in bone marrow, spleen, gastrointestine and testis of WBI mice. Our results demonstrated that sesamol and melatonin pre-treatment decreased the expression pattern of apoptotic proteins-ATM, p53, p21, Bax, cytochrome C, caspases-3 and caspases-9. The decrease of apoptotic proteins were associated with the increase of anti-apoptotic protein-Bcl-x and accordingly balance Bax/Bcl-x ratio. Thus, here it is hypothesized that part of the radioprotective effects of sesamol and melatonin may be due to the inhibition of expression pattern of ATM-dependent p53-mediated apoptotic proteins in bone marrow, spleen, gastrointestine, and testis of WBI mice.

Outcome of the PhD thesis: Four first authored research articles and eleven national and international abstracts.