Abstract:

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Title of the thesis: **Black Tea in Prevention of Arsenic Induced Skin Carcinogenesis: A Mechanistic Study**

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Groundwater contamination with inorganic arsenic (iAs) is a global problem. Chronic exposure to iAs, above the safe limit of 10µg/l, causes several health hazards, including cancer. Preliminary manifestations of chronic iAs exposure appears on the skin, which may develop into Squamous Cell Carcinoma (SCC). iAs promotes excess generation of Reactive Oxygen Species (ROS) causing DNA, protein, and lipid damage; it hinders antioxidant machinery, disrupts DNA Repair mechanism, promotes chronic inflammatory conditions, and induces epigenetic modulations, all contributing to carcinogenesis. iAs, by altering signalling pathways aid in Epithelial to Mesenchymal Transition (EMT), that plays a vital role in the etiology of skin cancer. ROS triggers a series of events leading to EMT, hence carcinogenesis. Drugs used to treat SCC are expensive with several toxic side effects. Non-toxic phytochemicals may be a solution to the problem. Black tea, a popular beverage and good antioxidant may be explored in quenching of iAs induced ROS and countering its associated adverse effects. The present study elucidates the effect of chronic iAs exposure, on Swiss albino mice (*in vivo*) and normal human skin keratinocytes HaCaT cells (*in vitro*). Ameliorative property of Black tea extract (BTE) in this regard has been explored. Co-carcinogenicity of iAs has been studied in *in vivo*, using 7,12-dimethylbenzanthracene (DMBA) as an initiator.

Histological analysis confirmed the development of invasive SCC of the skin, in Swiss albino mice, after 330 days of chronic iAs exposure. A group of mice which were administered BTE along with iAs, developed few dysplastic lesions during the study period. BTE was able to quench iAs induced excess ROS generation and counter DNA, protein and lipid damage in *in vivo* and *in vitro* model. Repair potential, expression of repair enzymes and activities of antioxidative enzyme were restored in the BTE group. Aberrant activities of inflammatory cytokines and NF-κB has been found to be modulated by BTE. A global hypomethylation was observed, of the H3K4 locus in iAs administered mice which was reversed in the BTE treated group. Results showed downregulation of H4K16ac, H3K4me3 and their acetyl and methyl transferases MYST1 and MLL1 respectively. Upregulation of H3K27me3 and H3K4me1 along with respective methyl transferases EZH2 and MLL3 was noted in the iAs treated mice. Loss of H4K16ac, H3K4me3 and gain of H3K27me3, H3K4me1 due to iAs appears to be reversed by intervention with BTE. JARID1B, a specific H3K4me3 demethylase, was found to be upregulated in the iAs treated mice. BTE failed to downregulate the elevated level of JARID1B, though prominent upregulation of H3K4me3 was found. It was hypothesized that, BTE might have deactivated the demethylating capability of JARID1B without modulating its expression. For a better understanding, in-silico docking studies had been performed. Results revealed that, theaflavin and theaflavin-3,3'-digallate, major components of BTE, were able to bind with the JmjC domain (ligand recognition and demethylating region) of JARID1B with high affinity. Molecular dynamical simulation studies confirmed the stability of these docking poses. Chronic iAs exposure also led to modulation of TGF-β pathway. Upregulated TGF-β transmitted its downstream signalling mainly by the non-canonical PI3K-AKT and MAPK pathways. However, BTE administration, resulted in downregulation of the signalling intermediates of PI3K-AKT, MAPK pathways. The signalling intermediates of the PI3K-AKT and MAPK pathways induced EMT and aided in cancer progression, therefore, downregulation of these molecules by BTE, may hint at its anti-cancer and chemopreventive ability. In *in vitro* model, induction of EMT in iAs exposed HaCaT cells had been observed, which were inhibited by BTE. Besides, BTE was found to inhibit the invasive and migratory transformations caused due to chronic iAs treatment, indicating at its anti-metastatic and anti-angiogenic properties.

BTE may ameliorate iAs induced skin carcinogenesis, both in Swiss albino mice and HaCaT cells, by quenching excess ROS generation, reversing epigenetic modulations, halting EMT and metastasis. Therefore, BTE can be an effective chemopreventive agent to counter iAs induced skin carcinogenesis.

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