Conclusions
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Mutations in the BRCA1 gene have profound importance in predisposing breast and ovarian cancer. BRCA1 germline mutations have been identified in nearly 50% cases of hereditary breast cancer and 80% of cases with both hereditary breast and ovarian cancers (HBOC). BRCA1 generally plays a role in breast cancer either due to its promoter hypermethylation or allele loss. Hence BRCA1 is also involved in sporadic tumors. Treatment modalities against BRCA1 mutated tumors had drawn less attention and an effective drug against them are lacking.

Standard chemotherapeutics have been developed that are used to target the repair deficient cancers, especially BRCA1 mutated breast and ovarian cancers. The emergence of PARP inhibitors against HR deficient tumors was possible by utilising the synthetic lethal concept, that were obtained from RNAi screen. Further clinical and preclinical trials indicated that the resistance caused by a subset of BRCA-less tumors to this novel therapeutics is due to the emergence of varied allelic alterations and reversal mutations. This caused the PARP inhibitors to stumble before BRCA deficient tumors. The development of novel therapeutic agents against BRCA mutated tumors is the need of the time due to the emergence of the cancer at a very young age. It had been proved that the member of the naphthaquinone family, Plumbagin has been effective against BRCA1 blocked tumors of ovarian origin. This was the rationale for using them in BRCA1 mutated breast cancers. The targeted efficacy of Plumbagin against BRCA mutated tumors are proved through cytotoxicity assay in the presence and absence of BRCA1 by a reconstitution based MTT assay. In continuation to the previous experimental data in genomically diverse cancer cells, Plumbagin induces the generation of reactive oxygen species in BRCA mutated breast cancer cells. This reactive oxygen species induces DSBs in DNA and subsequent downstream signalling by ATR/ATM kinases. This results in cell death that is an outcome of apoptotic phenomena elicited by Plumbagin. the combination of Plumbagin with PARP inhibitors facilitate the therapeutic repositioning of PARP inhibitors for effective inhibition of cancer cell growth. This study provides the molecular action of Plumbagin against BRCA1 mutated tumors and provide novel descriptors for Plumbagin action over its already existing pleiotropic character against cancer.
Most of the anticancer chemotherapeutic drugs that are broadly and successfully used today are DNA damaging agents. Targeting of DNA has been proven to cause relatively potent and selective destruction of tumor cells. However, the clinical potential of DNA-damaging agents is limited by the adverse side effects and increased risk of secondary cancers as consequences of the genotoxicity of these agents. The BRCA1 mutated tumors are more sensitive to DNA damaging agents. However, increased resistance are also showed by these cancers against standard DNA repair targeting therapeutics like PARP inhibitors. It was earlier reported that Plumbagin showed enhanced activity when combined with copper complexes (Chen et al., 2009). There is a need of new molecules that take advantage of the greater dependence of tumor cells on certain DNA-related processes, and eliminate adverse side effects including secondary cancer, by using compounds that bind to DNA. The anticancer effect of the copper complex of carbohydrazone having targeted effect against breast cancer cells was studied. The molecular pathway elicited by this copper chelated carbohydrazone (CS2) involved the induction of apoptosis by DNA double strand breaks. The targeted action of CS2 against BRCA1 deficient breast cancer cells is explained through its DNA interacting ability and topoisomerase inhibitory function. The combination of CS2 with Plumbagin provides a new dimension for the increased potency as an anticancer molecule at reduced IC₅₀ concentration than Plumbagin used alone in BRCA1 deficient cells.

The chemotherapeutic potential of Plumbagin as a single agent was tested in Brca1 knock out mouse models. Plumbagin induced apoptosis in mammary tumor of transgenic mouse generated from two different conditional strains. The mechanism of apoptosis takes place by inducing DSBs at the genomic level. The toxicity profile shown by Plumbagin in various tissues of transgenic mice studied, provided evidence for its safer disposition as a therapeutic lead in breast cancer research. In our study the concentration of Plumbagin administered were 2mg/ kg/ b.w. that was sixteen times less than carboplatin administration which was 32mg/ kg/ b.w. Additionally, Carboplatin induced tremendous side effects in humans including peripheral neuropathy, central neurotoxicity, nephrotoxicity, ototoxicity and even abnormal cardiovascular events. The oral bioavailability of Plumbagin was higher than that of Curcumin, while the plasma levels of Plumbagin have not yet been determined. As a future prospect, the micronuclease assay on the cultured human peripheral lymphocytes can be performed as this would provide a
discriminative evidence for the safety of Plumbagin in humans. The present study provides the foundation for the future use of Plumbagin in accurately scheduled clinical trials.