**PONDICHERRY UNIVERSITY**

**PUDUCHERRY – 605014**

**EXECUTIVE SUMMARY OF THE FINAL REPORT OF THE WORK DONE ON THE PROJECT**

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| 1. | Title of the Project | **Screening for molecular targets of anti-cancer drug using *Saccharomyces cerevisiae*** |
| 2. | Name and address of the Principal Investigator | Dr. Madhu Dyavaiah<br>Assistant Professor<br>Department of Biochemistry and Molecular Biology<br>Pondicherry University, Pondicherry - 605014 |
| 3. | Name and address of the Institution | Pondicherry University, Kalapet<br>Pondicherry - 605014 |
| 4. | UGC Approval letter no. and date | F.No.42-665/2013) (SR) dated 25/03/2013 |
| 5. | Date of implementation | 01/04/2013 |
| 6. | Tenure of the project | From 25-03-2013 to 31-03-2017 |
| 7. | Total grant allocated | Rs. 11,65,800 /- |
| 8. | Total grant received | Rs. 9,70,703/- |
| 9. | Objectives of the project | 1. Screening of yeast mutants strains under taxol stress.<br>2. Cell cycle analysis of yeast mutant strains after taxol treatment.<br>3. Analysis of DNA damage response transcripts and proteins expression level under taxol stress condition. |
| 10. | Whether the objectives were achieved? (Give details). | Yes |
| 11. | Achievements from the project | 1. New molecular targets of the anticancer drug, taxol were identified in this project using yeast gene deletion mutant strains.<br>2. These target genes are important players in oxidative stress response, DNA damage response and spindle assembly checkpoint pathways which are aberrant in cancer cells.<br>3. The findings from this project have a high clinical relevance as the cells deficient of these genes are highly sensitive to taxol. |
| 12. | Summary of findings (in 500 words) | Ever since taxol was isolated from *Taxus brevifolia,* passed clinical trials and has been successfully used |

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| | | as a first line chemotherapeutic drug in the treatment of many human cancers. Apart from stabilizing microtubules, taxol might also have other modes of inducing cytotoxicity by inducing ROS in the cancer cells in which specific oxidative stress management gene is impaired. Taxol might induce DNA damage in the cells with impaired DNA damage response (DDR). In addition, taxol may sensitize the cells with defective spindle assembly checkpoint (SAC) genes. This study shows that the cytotoxic property of taxol is attributed to its multifaceted mechanism of action. In this study, we have used yeast *Saccharomyces cerevisiae* gene deletion mutant strains of oxidative stress response, DNA repair and spindle assembly checkpoint pathways to identify new molecular targets of taxol other than tubulin. Among the yeast mutant strains screened, those belonging to these pathways were found sensitive to taxol in comparison with wildtype strain. We found that yeast oxidative stress response mutants (*sod1*Δ, *cta1*Δ and *tsa1*Δ), DDR mutant strains (*mre11*Δ, *sgs1*Δ, and *sub1*Δ) and SAC mutants (*bub1*Δ and *bub3*Δ) were highly sensitive to taxol treatment. Our screening results were further validated in solid growth medium. Because the antioxidant mutants were sensitive, we measured the levels of reactive oxygen species (ROS) levels induced by taxol by DCF-DA staining by fluorescence microscopy and spectrofluorometric methods. Our results indicate an increase in the level of ROS in yeast antioxidant mutant strains upon taxol treatment, compared to wildtype strain. Further, we observed apoptotic features in DDR as well as SAC mutant strains when exposed to taxol by nuclear staining using DAPI and acridine orange / ethidium bromide which suggests taxol may induce apoptotic death in the cells deficient of specific DNA repair and SAC genes. Growth curve experiment was also carried out with the exponentially growing yeast WT, antioxidant, DDR and SAC mutant cultures treated with or without taxol. The results showed a significant reduction in the growth rate of taxol treated yeast mutant cells in a time dependent manner, compared to wildtype. This suggests that taxol perhaps causes defects in the yeast cell cycle. In order to evaluate the effect of taxol on the oxidative stress response, DDR and SAC gene expression, we treated WT cells with taxol and the expression levels of oxidative stress response, DDR and SAC genes were analysed by quantitative real time PCR. An increase in the expression of these genes was observed in the yeast |

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| | | wildtype strain under taxol treatment. To evaluate how taxol affects the cell cycle progression of yeast SAC mutants, we treated both WT and a SAC mutant strain. A prolonged incubation of SAC mutants with taxol may induce irregular division of chromosomes leading to aneuploidy. Our results also suggest that an increase in the S-phase cell number may be due to taxol induced offence to DNA. Our findings present additional targets of taxol which has a high clinical relevance to sensitize the cells with specific molecular defects. |
| 13. | Contribution to the society (Give details) | Taxol is a first line chemotherapeutic drug used in the treatment of a wide variety of cancers. The results from this project suggest that the cells deficient of specific pathway genes in humans would be sensitive to taxol. These findings are relevant to selective targeting of the tumors with specific genetic defects in humans in order to reduce the undesirable side effects associated with the therapy. |
| 14. | Whether any PhD enrolled or produced out of the project? | No |
| 15. | No. of publications from the project | 03 Published, 01 communicated (Under revision) |

**Principal Investigator**

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