

## Acknowledgements

I am heartily thankful and owe my deepest gratitude to my supervisor, *Dr. Amit Kumar Mishra*, who has provided me the opportunity and support to pursue Ph.D. under his guidance at Indian Institute of Technology Jodhpur. I am grateful for his constant motivation and efforts without which I would not be able to complete my research work which I am presenting here. I am thankful to *Mr. Bharat Pareek, Dr. Deepak Chhangani, Arun Kumar Upadhyay, Vibhuti Joshi, Ribhav Mishra* and all other laboratory members who have helped me to smoothly fulfill various tasks throughout my research work associated with my thesis completion.

I would like to acknowledge all the scientists and professors who have helped me with research related materials and critical inputs. Special thanks to *Prof. Nihar Ranjan Jana, Prof. Koji Yamanaka* and *Dr. Ranbir Das*. I also thank my Ph.D. committee members *Dr. Meenu Chhabra, Dr. Sushmita Jha* and *Dr. Samanwita Pal* for their valuable suggestions and inputs at various stages during my Ph.D. work.

I pay my sincere and deepest gratitude to my Mother and Sisters for their unconditional support. In the absence of my Father *Late Dr. Mohd. Amanullah* they showed immense patience and endurance. I am indebted to my extended Family Members for their invaluable support and help. Special thanks to *Mohd. Saud, Mohd. Arif, Mohd. Yusuf, Mohd. Asif, Momin* and *Junaid*. I am grateful to my friend *Amitap Khandelwal* at IIT Jodhpur for his moral support and encouragement. I would like to thank all the Staff Members of Indian Institute of Technology Jodhpur and my funding agency, Ministry of Human Resource Development, Government of India, for their administrative and funding support.

Last but not the least; I would like to thank God, as I strongly believe that whatever he has done so far is best for me.

*Ayeman Amanullah*  
Ph.D. Student



## List of Figures

Figures	Title	page
1.1	Ubiquitin proteasome system and cellular proteostasis	2
2.1	Downstream effects of proteome disturbance	8
2.2	Schematic representation of various strategies to protect nascent polypeptide chains against misfolding	9
2.3	Diagrammatic representation of target mechanisms for faster clearance of multifactorial proteotoxic load generated by aberrant protein for therapeutic implication	15
3.1	Diclofenac exposure leads to enhanced buildup of ubiquitylated proteins	28
3.2	Diclofenac causes increased accumulation of ubiquitinated polyglutamine proteins	28
3.3	Diclofenac treatment leads to accumulation of ubiquitylated proteins around MTOC region	29
3.4	Diclofenac demonstrates <i>in silico</i> interaction	30
3.5	Diclofenac treatment exhibited apoptosis like characteristics in cells	31
3.6	Diclofenac inhibits purified proteasome activities	32
3.7	Diclofenac treatment resulted in inhibition of cellular proteasome activities in dose dependent manner	32
3.8	Diclofenac exposure resulted in inhibition of cellular proteasome activities in time dependent manner	33
3.9	Diclofenac application aggravates aggresome-like structures in cells	33
3.10	Diclofenac treatment reduces cell viability	34
3.11	Treatment with diclofenac increases level of proteasomal substrate I $\kappa$ B- $\alpha$ and reduces NF- $\kappa$ B activity	35
3.12	Diclofenac treatment decreases turnover of model proteasomal substrate	36
3.13	Diclofenac exposure showed increased accumulation of ubiquitylated proteins	37
3.14	Diclofenac treatment induces accumulation of proteasome targeted proteins 20S and p53	38
3.15	Diclofenac application induces accumulation of proteasome targeted proteins p27 and Ub	38
3.16	Diclofenac treatment induces accumulation of proteasome targeted proteins p21 and Bax	39
3.17	Diclofenac exposure induces accumulation of various proteasome targeted pro-apoptotic proteins	39
3.18	Diclofenac treatment leads to activation of apoptosis	40
3.19	Application of diclofenac leads to chromatin condensation and DNA fragmentation	41
3.20	Diclofenac treatment results in changes in the mitochondrial potential and induces cytochrome c release	42
3.21	Diclofenac exposure results in mitochondrial membrane depolarization and activates apoptosis signaling	43
4.1	Treatment of indomethacin generates apoptosis like effects	48
4.2	<i>In silico</i> interaction of indomethacin with proteasome subunits	48
4.3	The STD-NMR experiment showed that indomethacin interacts with proteasome	49
4.4	Treatment of indomethacin diminishes proteasome activity in concentration dependent manner	50
4.5	Treatment of indomethacin diminishes proteasome activity in time dependent manner	50
4.6	Treatment of indomethacin diminishes purified proteasome activity	51
4.7	Indomethacin exposure effects clearance of aggregate prone proteins	52
4.8	Indomethacin exposure leads to enhanced ubiquitylated proteins accumulation	53
4.9	Indomethacin exposure leads to increased accumulation of ubiquitylated proteins around MTOC region	54
4.10	Indomethacin exposure leads to ubiquitylated protein accumulation in time dependent manner	55
4.11	Indomethacin exposure leads to reduced cell viability	56
4.12	Treatment of indomethacin induces accumulation of proteasomal substrate	57
4.13	Application of indomethacin induces aggresomes like structure	58
4.14	Indomethacin treatment decreases turnover of proteasomal model substrate	59
4.15	Indomethacin accelerates accumulation of proteasomal substrates 20S, Ubiquitin and Bax	60
4.16	Indomethacin accelerates accumulation of proteasomal substrates p53, p27 and p21	60

<i>Figures</i>	<i>Title</i>	<i>page</i>
4.17	Indomethacin treatment induces accumulation of various proteasome targeted pro-apoptotic proteins	61
4.18	Indomethacin treatment reduces cell viability and induces senescence	62
4.19	Indomethacin treatment activates apoptosis	62
4.20	Indomethacin treatment leads to DNA fragmentation and neurite like outgrowth	63
4.21	Impairment of mitochondrial function after indomethacin treatment and release of cytochrome c	64
4.22	Indomethacin treatment leads to mitochondrial membrane depolarization	64
4.23	Indomethacin treatment leads to accumulation of ubiquitylated proteins in mitochondrial membrane	65

## List of Tables

<i>Table</i>	<i>Title</i>	<i>page</i>
A.1	<i>List of Antibodies Used in the Study</i>	71
A.2	<i>List of Expression Plasmids Used in the Study</i>	72

## List of Symbols

<i>Symbol</i>	<i>Description</i>
$\alpha$	Alpha
$\beta$	Beta
$\gamma$	Gamma
$\mu$	Micro
$^{\circ}$	Degree
m	Milli
M	Molar
C	Celsius
g	Gravitational force
Hz	Hertz
ppm	Parts per million
hrs	Hours
L	Litre
nm	Nano meter
$\Delta\psi_m$	Change in mitochondrial membrane potential

## List of Abbreviations

<i>Abbreviation</i>	<i>Full form</i>
AMC	7-amino-4-methylcoumarin
ATG	Autophagy Related Gene
ATP	Adenosine Triphosphate
Bax	Bcl2 Associated X Protein
CAP	Chaperone Assisted Proteasomal Degradation
CASA	Chaperone Assisted Selective Autophagy
CDK	Cyclin-Dependent Kinase
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CMA	Chaperone Mediated Autophagy
COX	Cyclooxygenase
d1EGFP	Destabilized Enhanced Green Fluorescent Protein
DAPI	4',6-diamidino-2-phenylindole
Dic	Diclofenac
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
EGFP	Enhanced Green Fluorescent Protein
ERAD	Endoplasmic Reticulum associated Degradation
FACS	Fluorescence Assisted Cell Sorter
FBS	Fetal Bovine Serum
FID	Free Induction Decays
FITC	Fluorescence Isothiocyanate
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HA	Haemagglutinin
HDQ	Huntington's Disease Glutamine
HEPES	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic Acid
HRP	Horseradish Peroxidase
HSF1	Heat Shock Transcription Factor 1
Hsp	Heat Shock Protein
Indo	Indomethacin
JC-1	5,5', 6,6'-Tetrachloro-1,1',3,3' Tetraethylbenzimidazolcarbocyanine Iodide
LAR-II	Luciferase assay reagent-II
MTOC	Microtubule Organizing Center
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NAC	Nascent Polypeptide-Associated Complex
NAC	N-Acetyl Cysteine
NDDs	Neurodegenerative Diseases
Noc	Nocodazole
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
PBS	Phosphate Buffer Saline
PDB	Protein Data Base
PGPH	Peptidylglutamyl Peptide Hydrolyzing
PI	Propidium Iodide
PQC	Protein Quality Control

QC	Quality Control
RAC	Ribosome-Associated Complex
RLU	Relative Luminescence Unit
SDS	Sodium Dodecyl Sulfate
STD-NMR	Saturation Transfer Difference Nuclear Magnetic Resonance
TBST	Tris Buffer Saline-with Tween 20
TUNEL	Terminal Deoxynucleotidyl-dUTP Nick End Labeling
Ub	Ubiquitin
UPR	Unfolded Protein Response
UPS	Ubiquitin Proteasome System
VDAC	Voltage Dependent Anion Channel