Table of Contents

Chapters	Title	Page No.
Chapter 1	Review of Literature.	1
1.	Introduction	2
1.1	Heavy metals	2
1.2	Mechanism of action	2
1.3	Sources of hazardous metals and minerals	3
1.4	Cadmium (Cd)	4
1.4.1	Toxicokinetics	4
1.4.2	Absorption	5
1.4.3	Distribution	5
1.4.4	Health effects	6
1.5	Lead (Pb)	7
1.5.1	Toxicokinetics	7
1.5.2	Absorption	8
1.5.3	Distribution	8
1.5.4	Health effects	8
1.5.5	Pb induced immunotoxicity	10
1.6	Cd and Pb coexposure	11
1.7	Arsenic (As)	13
1.7.1	Toxicokinetics	13
1.7.2	Absorption	14
1.7.3	Distribution	14
1.7.4	Health effects	14
1.7.5	Biotransformation of As in humans	15
1.7.6	Arsenic resistance in Prokaryotes	17
1.8	Dislipidemia	18
1.9	Metallothionein	19
1.10	Combination therapy: chelators, antioxidants, probiotics, prebiotics, synbiotics	20
1.10.1	Chelation: concept	20
1.10.2	Antioxidants with chelators	21
1.10.3	PQQ (Antioxidant)	22
1.10.4	Probiotics	27
1.10.5	Prebiotics and SCFAs (Short chain fatty acids)	34
1.10.6	Synbiotics	38
1.11	Gut microbiota: Novel detoxification strategy for heavy metals	38
1.11.1	Bioremediation by bacteria in environment	38
1.11.2	Human tolerance to metals	39
1.11.3	Gut microbiota: Mechanisms Of action	40
1.11.4	Sequestering heavy metals by probiotic bacteria	40
1.11.5	Probiotics against Arsenic toxicity	41
1.11.6	Probiotics against Lead and Cadmium toxicity	41
1.11.7	Designer probiotics	42

1.12	*E. coli* Nissle as potential probiotic strain	45
1.12.1	Genetically modified *E. coli* Nissle as therapy against heavy metal toxicity	47
Chapter 2	Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing NADH insensitive citrate synthase-sodium dependent citrate transporter (*csYF-citC*) and pyrroloquinoline quinone (*pqq*) gene cluster in amelioration of cadmium induced toxicity in rats.	51
2.1	Introduction	52
2.2	Materials and methods	54
2.2.1	Animals	54
2.2.2	Cloning	54
2.2.3	Characterization of *Ec*N transformants producing PQQ and citric acid	55
2.2.4	Bacterial strains and culture conditions	56
2.2.5	Experimental design	56
2.2.6	Preparation of tissue homogenates	57
2.2.7	Biochemical Assays	57
2.2.8	ALT, AST, ALP, total bilirubin, urea and creatinine	57
2.2.9	Histopathological changes	57
2.2.10	Cd levels	57
2.2.11	Statistical analysis	57
2.3	Results	58
2.3.1	Cloning and characterization of *Ec*N-2 transformants	58
2.3.2	Effect of *Ec*N-2 transformants against Cd induced liver and kidney damage in rats	60
2.3.3	PQQ quantification from faeces and liver	62
2.3.4	Cd Estimation	62
2.3.5	Histological analysis	63
2.4	Discussion and Conclusion	64
Chapter 3	Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad*) and pyrroloquinoline quinone (*pqq*) gene cluster in amelioration of cadmium induced toxicity in rats.	67
3.1	Introduction	68
3.2	Materials and methods	68
3.2.1	Animals	68
3.2.2	Cloning	70
3.2.3	Characterization of *Ec*N transformants producing PQQ, gluconic acid and 2-Ketogluconic acid	70
3.2.4	Bacterial Strains and Culture Conditions	70
3.2.5	Experimental Design	70
3.2.6	Preparation of Tissue Homogenates	71
3.2.7	Biochemical Assays	71
3.2.8	ALT, AST, ALP, Urea,Creatinine	71
3.2.9	Histopathological Changes	71
3.2.10	Statistical Analysis	71

3.3	Results	71
3.3.1	Characterization of PQQ, gluconic and 2-ketogluconic acids secretion by *Ec*N transformants	71
3.3.2	Effect of *Ec*N-23 against Cd induced liver and kidney damage in rats	72
3.3.3	Histopathological damage	75
3.4	Discussion and Conclusion	76
Chapter 4	Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad)* and pyrroloquinoline quinone (*pqq)* gene cluster in amelioration of LPS/GalN induced damage in lead treated rats.	78
4.1	Introduction	79
4.2.	Methods and Materials	79
4.2.1	Animals	79
4.2.2	Bacterial Strains and Culture Conditions	79
4.2.3	Experimental Design	80
4.2.4	Preparation of Tissue Homogenates	80
4.2.5	Biochemical Assays	80
4.2.6	Histopathological Changes	81
4.2.7	mRNA Expression and Quantitative Reverse Transcription PCR	81
4.2.8	Statistical Analysis	81
4.3	Results	81
4.3.1	Effect of *Ec*N-23 against LPS/GalN induced immunotoxicity in Pb treated rats	81
4.3.2	Histopathological damage	85
4.4	Discussion and Conclusion	86
Chapter 5	Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing gluconate dehydrogenase (*gad)* and pyrroloquinoline quinone (*pqq)* gene cluster against long term coexposure of cadmium and lead in rats.	88
5.1	Introduction	89
5.2	Materials and Methods	89
5.2.1	Animals	89
5.2.2	Bacterial Strains and Culture Conditions	89
5.2.3	Experimental Design	90
5.2.4	Preparation of Tissue Homogenates	90
5.2.5	Biochemical Assays	90
5.2.6	ALT, AST, ALP, Total Bilirubin, Urea,Creatinine, Ca, Mg, Zn, Fe levels and Blood lipid estimaton	90
5.2.7	Histopathological Changes	90
5.2.8	mRNA Expression and Quantitative Reverse Transcription PCR	91
5.2.9	Metal Determination	91
5.2.10	Statistical Analysis	91
5.3	Results	91
5.3.1	Effect of *Ec*N-23 against long term coexposure of Cd and	91

	Pb in rats	
5.3.2	Histopathological changes	96
5.4	Discussion and Conclusion	97
Chapter 6	Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing As(III) S-adenosylmethionine (SAM) methlyltransferase (*arsM*) and pyrroloquinoline quinone (*pqq)* gene cluster in amelioration of arsenic induced toxicity in rats.	101
6.1	Introduction	102
6.2	Methods and Materials	103
6.2.1	Animals	103
6.2.2	Cloning	103
6.2.3	Characterization of *Ec*N transformants	105
6.2.4	Bacterial Strains and Culture Conditions	105
6.2.5	Experimental Design	105
6.2.6	Preparation of Tissue Homogenates	105
6.2.7	Biochemical Assays	106
6.2.8	ALT, AST, ALP, Urea, Creatinine and Blood lipid estimaton	106
6.2.9	Histopathological Changes	106
6.2.10	As levels	106
6.2.11	Statistical Analysis	106
6.3	Results	107
6.3.1	Cloning and characterization of *Ec*N-2 transformants	107
6.3.2	Effect of *Ec*N-2 transformants against As induced liver and kidney damage in rats	107
6.3.3	Effect of *Ec*N-2 transformants against As induced dislipidemia in rats	109
6.3.4	PQQ Quantification from faeces and liver	109
6.3.5	SCHAs Quantification from faeces	114
6.3.6	As Estimation	114
6.3.7	Histological analysis	114
6.4	Discussion and Conclusion	115
7	Summary	120
8	References	125