Chapter V

Summary and Conclusions

Diet plays an important role in the progression of the metabolic disorders through affecting the gut microbiota colonization. HSD feeding in the wistar rats for around 12 weeks had showed to increase the body weight, fasting blood glucose, serum triglycerides and serum cholesterol levels as compared with the CD group. HSD had also showed elevated CRP level, which is a positive marker for the inflammation. HSD feeding had showed detrimental effects on the liver through increasing the liver lipid parameters as well as liver inflammatory enzymes level compared to the CD group. Analysis of the gut dominant microbial phyla and genera from fecal samples of the experimental animals using qPCR had revealed the altered gut microbial profiling in HSD fed rats as compared to the CD animals. HSD fed rats had showed an increase in the Bacteroides and Proteobacteria population with a reduction in the Firmicutes at the phyla levels. In addition, it had also decreased the *Bifidobacteria* and *Clostridia* numbers, while increased *Escherichia* population at genus level compared to the CD group. An increased Gram negative Proteobacteria and Bacteroidetes phyla along with an *Escherichia* genus following an HSD feeding had found to be positively correlated with an increased serum LPS levels. An increased serum LPS following an HSD administration had created the metabolic endotoxemia in the gut. LPS is an important ligand for the PRRs, such as TLR4 and NLR1. Thus, HSD group of animals had found to increase the mRNA expression of TLR4 and NLR1 in proximal small intestine and colonic tissue as compared to the CD group, which in turn activated the NF-κB. An increase in the PRRs expression by their ligands phosphorylates the Iκβ on two critical serine residues. This modification allows their polyubiquitination and destruction by the proteasome.

As a consequence, free NF-κB enters the nucleus and activates transcription of a variety of genes participating in the inflammatory response. The mRNA expression of inflammatory cytokines, such as TNF-α and IL-6 was found to be significantly increased in the liver, colon and proximal small intestine of the HSD group compared to the CD group. Proinflammatory cytokines can downregulate the IRS signaling pathway to progress the IR. Gut microbiota also play an important role in the production of SCFAs through fermentation of the complex non-digestible carbohydrates. The major alteration in the level of three SCFAs, i.e acetate, propionate and butyrate had been observed from the fecal samples of the HSD rats. The amount of acetate was found to be increased, while butyrate and propionate were found to be decreased in the HSD group of animals compared to the CD group. SCFAs participate in the progression or prevention of diabetes through GPCRs pathways. HSD fed animals had shown significant decrease in the mRNA expression of GPR43 compared to CD group. It had also shown to decrease the expression of incretin, such as GLP-1and glucose transporter GLUT4 in the colonic tissue compared to the CD group. The decrease in the GLP-1 and GLUT4 might had reduced the glucose uptake and found to develop the IR. The HSD induction for the 12 weeks of the period has shown decrease in the BSH enzyme bacterial copy numbers as compared with the CD group, while cefdinir and chitosan administration has increased the BSH enzyme copy number as compared with HSD control.

The gut microbiota modulation by spectrum specific antibiotics in their crude form had shown divergent effects on the HSD mediated IR at physiological, biochemical and inflammatory levels. Linezolid (Gram positive spectrum) administration along with HSD had less aggravated the IR compared with HSD control through increase in the Gram negative microbial population, i.e. Bacteroides and Proteobacteria, while decrease in the Firmicutes population in the colon. Cefdinir (Gram negative spectrum) administration in HSD rats had demonstrated improved insulin sensitivity through decreasing the gut microbes derived LPS in the colon. Significant reduction in the LPS had decreased the mRNA expression of TLR4 and NLR1, which had further decreased the NF-κB induced expression of inflammatory cytokines in the liver, proximal small intestine and colon. Cefdinir administration along with an HSD had increased the population of major SCFAs producing bacterial phyla, i.e. Firmicutes in the colon.

The gut microbiota modulation using cefdinir and chitosan had also increased population of *Clostridia* and *Bifidobacteria* in the colon, which have an important role in the production of butyrate. This observation can be correlated with an increased the amount of the butyrate in the cefdinir receiving animals compared to HSD control. Cefdinir administration in HSD had found to increase the mRNA expression of GPR43, GLP-1 and GLUT4 as compared with HSD control. It can be concluded that cefdinir administration in HSD rats had improved the insulin sensitivity through gut microbiota mediated inflammatory and metabolic pathways. Chitosan administration along with an HSD had increased the probiotic population, i.e. *Lactobacilli* and *Bifidobacteria* in the colon, which had shown to increase the butyrate levels in the gut. Chitosan had prevented the progression of HSD induced diabetes potentially through gut microbiota alteration.

The targeted antibiotics delivery system can overcome several limitations of the conventional system and enhance their therapeutic efficacy. An enteric coated antibiotics microspheres can deliver the drug at the target site with the appropriate dose to enhance the maximum therapeutic efficacy. Enteric coating of the antibiotics can prevent the premature drug release at upper gastrointestinal tract to reduce the toxicity, while microspheres formulation of antibiotics can enhance their bioavaibility through increasing the surface area. The EL and ES coated linezolid as well as cefdinir microspheres were formulated for their targeted delivery to proximal small intestine and colon respectively through solvent evaporation method. They had displayed the smooth and spherical surface morphology with particle size in range of 10-40 μM. The EL coated antibiotics microspheres had prevented the premature drug release in stomach and released it in the proximal small intestinal area (Duodenum or Jejunum), while ES coated microspheres had prevented the drug release at stomach and proximal small intestine to release them completely at the colonic region. Enteric coated antibiotics microspheres had shown higher efficiency in altering the microbial population of HSD as compared with crude antibiotics.

The ES coated linezolid microspheres administration in HSD had increased the fasting glucose, body weight, TG, TC, CRP levels as compared with HSD control and crude linezolid administrated HSD group. However, EL coated linezolid administration in HSD had not shown any significant effects on body weight, fasting glucose and various other parameters as compared

with HSD control. ES coated cefdinir microspheres administration in HSD rats had shown to decrease the fasting glucose, TG, TC, CRP and body weight gain compared with HSD control and crude cefdinir treated HSD group.ES coated cefdinir supplementation along with an HSD had significantly reduced the colonic Bacteroides and Proteobacteria population, thus reduced the serum LPS level compared to crude cefdinir treated HSD and HSD control. The colon and liver of these groups of animals had showed significant decrease in the mRNA expression of the proinflammatory cytokines, such as TNF-α and IL-6 compared with HSD control. ES coated cefdinir microspheres administration in HSD had significantly increased the insulin sensitivity as compared to crude cefdinir administrated HSD group. The EL coated cefdinir microspheres had not showed any significant effects on reducing the inflammation and improving the insulin sensitivity.

In conclusion, cefdinir (Gram negative specific) and chitosan had exhibited interesting diabetic preventive properties in HSD induced diabetic rats. They had prevented the progression of insulin resistance through HSD mediated gut microflora dysbiosis. They had decreased the Proteobacteria and Bacteroidetes population, which had decreased the inflammation. This may have lead to the activation of the Insulin receptor signalling (IRS) by decreasing the expression of the pro-inflammatory cytokines. They had further increased the amount of the major fermentative bacteria in the gut i.e *Bifidobacteria* and *Clostridia,* which had found to increase the amount of butyrate in the colon. Cefdinir had prevented downregulation of the SCFAs receptor expression i.e GPR43. Downstream signaling pathways of the GPCRs induced the release of incretin i.e GLP-1 which had increased the glucose uptake through GLUT4 and increased the insulin sensitivity. The proximal small intestinal microflora have major role in the absorption of the nutrients, while colonic microflora have major role in the anaerobic fermentation of the non-digestible carbohydrates. Moreover, microbial density and diversity are much higher in the colon compared to proximal small intestine. The colon targeted cefdinir administration had shown higher efficiency for the prevention of the HSD induced IR as compared to proximal small intestinal targeted delivery. The colonic microflora have very important role in the maintenance of human health through production of microbial metabolites. The selective manipulation of the

colonic microbiota using spectrum specific antibiotics and prebiotic can be an emerging therapeutic strategy for the prevention of IR.