6. SUMMARY AND CONCLUSION

The present study is aimed to determine whether TX administration ameliorates insulin resistance-related cardiac changes in a mice model of MS. Adult male *Mus musculus* mice of body weight 25-30 g were grouped into four and fed either control diet or HFFD for 60 days. On the 16th day each group was divided into two and treated or untreated with TX (150 mg/kg bw, p.o) for the next 45 days. Mice fed HFFD showed most of the phenotypic characteristics of MS like obesity, insulin resistance, hyperinsulinemia, dyslipidemia, and hypertension. HFFD feeding impaired insulin signaling and glucose uptake and utilization. This was verified by the decreased activation of insulin-stimulated IRS1-PI3K-Akt pathway and GLUT-4 translocation in the heart. Due to cellular insensitivity to insulin, decreased FA oxidation and increased lipid accumulation were observed. HFFD feeding caused oxidative stress in the cardiac tissue which was confirmed by increased ROS production, NADPH oxidase, lipid peroxidation and protein oxidation products and decreased levels of cardiac antioxidants.

ROS-mediated oxidative damage to mitochondria resulted in reduced efficiency of the electron transport chain, fall in uncoupling proteins -2 and -3, membrane potential generation, reduction in mitochondrial density and mitochondrial biogenesis, alteration in mitochondrial dynamics with a rise in mitochondrial calcium levels.

Metabolic changes, insulin resistance and mitochondrial dysfunction are documented to be the mechanisms for the development of cardiac disease. HFFD consumption for 60 days led to increased levels of cardiac function markers and ECG pattern resembling arrhythmia. Left ventricular remodeling and fibrotic changes were observed together with activation of proapoptotic proteins.

TX supplementation improved insulin sensitivity, increased GLUT-4 translocation and attenuated the risk factors of CVD such as dyslipidemia,
hyperinsulinemia, hypertension and impaired glucose tolerance. TX administration showed lipid-lowering effect through upregulation of PPAR-α resulting in SREBP-1c reduction thereby regulating lipid metabolism. TX was also found to activate AMPK phosphorylation which targets PPAR-α and inhibit SREBP-1c transcriptional activity.

Increased glucose availability to the heart by TX minimized structural and functional changes of the heart. TX treatment significantly reduced left ventricular/cardiac hypertrophy and ECG abnormalities and also improved cardiac function. TX diminished collagen levels and prevented the process of myocardial fibrosis. This was achieved by normalizing TGF-β1 and MMP-TIMP balance. TX, being an antioxidant attenuated the diet-induced changes by reducing oxidative stress in the heart. TX treatment markedly reduced lipid peroxidation markers and protein carbonyl levels and enhanced antioxidant levels. Further, the total antioxidant capacity of animals measured by FRAP assay was improved after TX treatment signifying its anti-oxidant power and cytoprotective effect. Our findings present new data that TX exerts its cardioprotective effect through suppressing the activity of NADPH oxidase p22phox subunit and thereby improves cardiac function. TX administration normalized mitochondrial function and increased mitochondrial content and biogenesis through PGC-1α signaling and attenuated mitochondria-mediated apoptosis.

From the results, it can be concluded that feeding HFFD caused insulin resistance, metabolic modulation and oxidative stress in heart. These are the underlying mechanisms that promote hypertrophic remodeling, mitochondrial dysfunction and cardiac damage. TX could attenuate these changes by lipid-lowering, insulin-sensitizing, cardioprotective and antifibrogenic effects. These findings together with its
safety and tolerability profile imply that TX could be a good candidate for
the management of cardiac diseases associated with the MS. The
pathological sequence of events as the outcome of HFFD feeding and the
potential effects of TX are presented in Fig. 40. The new data on TX could
pave way for additional studies on the mechanism of action of TX.
**Fig. 40** HFFD-induced pathological changes and potential sites of action of TX. HFFD results in impairment in insulin signaling, oxidative stress and dysregulation in lipid metabolism. HFFD-induced oxidative stress (ROS) in turn promotes cardiac dysfunction, hypertrophy and remodeling and damages the mitochondria leading to apoptosis. TX administration improves insulin signaling, regulates lipid metabolism and attenuates the cardiac consequences of HFFD. ECM- Extracellular matrix; HFFD- High fat, high fructose diet; IGT- Impaired glucose tolerance; IR- Insulin resistance; ROS- Reactive oxygen species; TX- Troxerutin.