7. SUMMARY

The comparative study of pioglitazone (insulin sensitizer), quercetin (hepatoprotectant and antioxidant) and hydroxy citric acid (hypolipidemic and anti obesity agent) were carried out in modified experimental model of NASH in rats. In this study, by analyzing various biochemical, histopathological, immuno histochemical, PCR studies etc, we can understand the mechanism of drugs showing better therapeutic action on NASH.

The findings of this research study can be summarized as follows:

7.1. Experimental NASH model in rats, which mimics NASH in humans, was produced by feeding high fat diet ad libitum for 8 weeks, confirmed by the histopathological studies and supported by the scanning electron microscopy studies.

7.2. The hepatoprotective action of the three drugs of choice (pioglitazone, quercetin and hydroxy citric acid) was observed by treating the rats with high fat diet for 4 weeks, then feeding them high fat diet simultaneously with intra gastric administration of pioglitazone, quercetin and hydroxy citric acid, for another 4 weeks. The hepatoprotective action of pioglitazone, quercetin and hydroxy citric acid was confirmed by biochemical parameters and NASH biomarkers.

7.3. ALT, AST, GGT and LDH levels are fluctuated in experimentally induced NASH group when compared to rats in control group. The
ratio between ALT and AST has also been found to have predictive value. The protective effect of quercetin on experimental NASH showed more significant reduction of these liver enzymes in comparison with pioglitazone and hydroxy citric acid therapy.

7.4. A significant increase in the levels of total bilirubin, creatinine, urea, uric acid and glucose was noticed in experimental NASH group whereas in quercetin, hydroxy citric acid and pioglitazone treated groups normalize these parameters.

7.5. Serum lipid profile such as total cholesterol, free cholesterol, esterified cholesterol, phospholipids, triglycerides and free fatty acids levels raised significantly in experimental NASH. Hydroxy citric acid and pioglitazone showed much reduction in lipid levels when compared to quercetin treated groups.

7.6. Significant elevation of lipoproteins such as HDL, LDL, VLDL, HDL:LDL, TC:HDL were observed in experimental NASH, indicating that the disease progression was directly proportional to the lipoprotein levels. Protective effect of quercetin and hydroxy citric acid was noticed significantly when compared to experimental NASH. Significant reduction was also observed in pioglitazone treated rats.

7.7. The levels of non-enzymatic and enzymatic antioxidants were assessed in experimental NASH rats, and found to be decreased. The antioxidant property of quercetin and hydroxy citric acid could
be more beneficial in treatment of NASH when compared to pioglitazone treated rats.

7.8. The imbalanced production of pro- and anti inflamatory adipokines secreted from fat contributes to the pathogenesis of NASH was observed. Adiponectin is an important adipokine decreased significantly, whereas the levels of hyaluronic acid and leptin were increased significantly in experimentally induced NASH group, compared to control group. Quercetin appeared to protect against liver injury and reverse the levels of hyaluronic acid, leptin and adiponectin towards normal. The same protective effect was observed in pioglitazone treated groups and hydroxy citric acid significantly.

7.9. TNF-α and MPO have long been recognized for their pro-inflamatory properties and their role in NASH progression was clearly established. TNF-α and MPO were highly expressed in experimentally induced NASH group, compared to control group. Quercetin and pioglitazone offers protection against NASH by ameliorating the inflammation (hepatitis), a principle and key feature of NASH, whereas hydroxy citric acid offers very little protection against NASH.

7.10. Quantitative real-time polymerase chain reaction (RT-PCR) analysis of vascular endothelial growth factor (VEGF) messenger RNA (VEGF mRNA) was analyzed in all the groups. Enhanced expression of VEGF mRNA was detected in experimental NASH
group whereas reduced expression of VEGF mRNA was observed in quercetin, pioglitazone and hydroxy citric acid treated groups. Quercetin showed an effective inhibition of VEGF mRNA expression and perhaps only smaller inhibition of VEGF mRNA level seen in hydroxy citric acid and pioglitazone treated rats.

7.11. Cytochrome P450 2E1 (CYP2E1) enzyme levels in liver was detected by immunoblot analysis. Increased expression of this enzyme is involved in the pathogenesis of NASH was observed through the generation of oxidative stress. Reduced expression of functional CYP2E1 protein was observed in quercetin, pioglitazone and hydroxy citric acid treated groups.

7.12. Cytokeratin-18 (CK-18), a potential biomarker for NASH was detected by immunohistochemical study. Increased expression of CK-18 was observed in experimentally induced NASH than in control group. The over expressed CK- 18 levels in NASH were markedly reduced by treatment with pioglitazone, quercetin and HCA suggesting their role to prevent cell death. The maximum significant protection was shown against NASH by quercetin compared to pioglitazone and HCA. Quercetin showed very less expression of CK-18 when compared to pioglitazone & hydroxy citric acid treated groups.

7.13. Noninvasive panels of serological markers have been developed to evaluate the presence of steatosis and hepatic necro-inflammation to avoid liver biopsy.
7.14. Tissue polypeptide specific antigen (TPSA) has recently been proposed as diagnostic marker of apoptosis in NASH and elevated levels were observed in experimental NASH when compared to control group, whereas TPSA was not expressed in alcoholic liver disease (ALD).

Thus, the results of the present investigation have fulfilled the objective and scope of this study.

CONCLUSION

NASH can be cured by reducing the oxidative stress and free radical production. It is earnestly hoped that the results obtained have opened up newer horizons of research in the area of NASH and its management by quercetin, pioglitazone and hydroxy citric acid. Present study conclusively demonstrated that quercetin, possessing potent radical scavenging, antioxidant, hepatoprotective and anti-inflammatory properties, could be safely developed as hepatoprotective drug for NASH. Future studies are required for clinical trials to try this protective mechanism of quercetin for NASH suffering patients.