7. Summary and Conclusions

7.1. Summary

The present studies were designed to systematically evaluate the efficacy and safety of some of the medicinal plant extracts for their potential in management of diabetic retinopathy in STZ induced type-1 and type-2 diabetic rats. The selected plants included *Momordica charantia* (MC), *Boerhavia diffusa* (BD), *Eugenia jambolana* (EJ), *Tinospora cordifolia* (TC) and polyherbal combination (PHC) of these drugs. Various physical, biochemical and histological parameters were monitored to assess the potential of experimental drugs. Anterior and posterior segment photographs were taken using slit lamp and fundus camera respectively.

**Summary of Diabetes Type 1 Prevention Study**

1. The streptozotocin induced diabetic rat model was standardized for type I diabetes at the dose of 45 mg/kg (i.p.) for the development of diabetic retinopathy.

2. *Momordica charantia, Boerhavia diffusa, Eugenia jambolana* and *Tinospora cordifolia* along with their combination (PHC) have all shown significant antihyperglycemic effect and also reduced HbA1c in STZ induced diabetes model. Polyherbal combination (PHC) showed maximum reduction. Among individual drugs best antihyperglycemic activity was seen in *M. charantia*, followed by *B. diffusa, T. cordifolia* and *E. jambolana*.

3. The experimental drugs significantly rectified the loss in body weight as compared to diabetic control group (p<0.0001).

4. Untreated diabetic animals showed maximum consumption of food and water as compared to normal group (p<0.0001). The consumption was significantly lesser in all the treated groups.
5. The drugs showed protection against dilatation of retinal vessels. The vessel diameter was significantly reduced in all the treatment groups as compared to that of diabetic control group.

6. Severe cataractous changes developed in anterior segments of diabetic control animals, which were, however protected in the drug treated animals.

7. Levels of anti-inflammatory marker TNF-α markedly increased in diabetic control retinae. PHC and M. charantia showed appreciable decrease (p<0.001) in its level. Treatment with B. diffusa led to moderate reduction (p<0.01), while only a mild reduction (p<0.05) in TNF-α level was shown by E. jambolana.

8. Cytokine IL-1β steeply upregulated in diabetic control group (p<0.0001). The drug extracts PHC, MC, BD and TC showed a good attenuation (p<0.0001) and EJ a moderate attenuation (p<0.001) in retinal level of IL-1β.

9. PHC, M. charantia and B. diffusa offered significantly high protection (p<0.0001) against the increase in VEGF, the angiogenic marker. T. cordifolia also exhibited a moderate protection for the same (p<0.001), however only a mild protection was provided by E. jambolana (p<0.05).

10. All the drug extracts significantly prevented the increase in retinal level of protein kinase-C (PKC) as compared to diabetic control group. Significance value for prevention against PKC rise due to treatment with PHC, M. charantia, B. diffusa and T. cordifolia, was p<0.0001 and due to E. jambolana it was p<0.001.

11. The drug extracts offered significant positive modulation of antioxidant reduced GSH in diabetic rat retina. Maximum antioxidant activity was restored by PHC (p<0.0001), while other drugs showed moderate modulation (p<0.001).

12. Activity of antioxidant enzyme, catalase, was best prevented by PHC (p<0.0001), followed by M. charantia (p<0.001). Other drugs, i.e., B. diffusa, T. cordifolia, and E. jambolana showed only a mild action (p<0.01) in preventing the antioxidant activity of the enzyme.
13. Histopathological findings confirmed that the experimental drugs prevented degenerative changes not only in retinal vasculature but also in other organs like kidney and pancreas, thus providing protection against other diabetic complications along with diabetic retinopathy.

14. PHC showed best preventive effects followed by MC > BD > TC > EJ

**Summary of Diabetes Type 2 Prevention Study**

1. The streptozotocin model for diabetic retinopathy due to type 2 diabetes was standardized at the STZ dose of 60 mg/kg (i.p.), injected to two days old pups.

2. The study drugs *Momordica charantia*, *Boerhavia diffusa*, *Eugenia jambolana*, *Tinospora cordifolia* and their combination (PHC) have all shown significant anti-hyperglycemic effect and also reduced HbA1c in a span of 24 weeks of study. PHC showed maximum effect followed by *M. charantia*, *B. diffusa*, *T. cordifolia* and *E. jambolana* respectively.

3. Significant rectification in loss in body weight was provided by the experimental drugs in treatment group as compared to untreated diabetic control group (p<0.0001).

4. Food and water consumption in the treatment groups was comparatively lesser than that in diabetic control group.

5. The vessel diameter in diabetic group significantly increased as compared to normal group. The experimental drugs exhibited preventive effect on vessel dilation.

6. Development of cataractous changes was observed in anterior segments of diabetic control animals. However, cataract development and lens deformity was prevented in the drug treated groups.

7. Retinal TNF-α level increased significantly (p<0.001) in diabetic control group as compared to normal control. Treatment with PHC provided appreciable
attenuation of this anti-inflammatory marker (p<0.001). *M. charantia* moderately reduced its level in retina (p<0.01), while only mild effect was shown by *B. diffusa* (p<0.05). However, *T. cordifolia* and *E. jambolana* failed to show any significant decrease in the level of TNF-α in comparison to diabetic control levels (p>0.05).

8. Level of cytokine IL-1β significantly upregulated in diabetic control group (p<0.001). The drug extracts PHC, *M. charantia*, *B. diffusa* and *T. cordifolia* showed an appreciable attenuation in its level (p<0.001). *E. jambolana* provided only a mild reduction in the cytokine level as compared to diabetic control (p<0.05).

9. Angiogenic marker VEGF, the key element of DR, was significantly reduced by the herbal extracts in all the treatment (p<0.001) as compared to diabetic control group.

10. Significantly high protection against the increase in retinal PKC level was provided by PHC, *M. charantia* and *B. diffusa* (p<0.001). *T. cordifolia* led to only a moderate reduction of PKC level (p<0.01). However *E. jambolana* failed to achieve significant reduction of PKC in retina (p>0.05).

11. The herbal extracts PHC, *M. charantia* and *B. diffusa* provided a strong defense (p<0.001) against the depletion of antioxidant glutathione (GSH). *T. cordifolia* was able to provide moderate protection (p<0.01), however, *E. jambolana* showed only a mild action (p<0.05) in restoration of GSH activity.

12. Activity of catalase enzyme, was well protected by PHC and *M. charantia* (p<0.001). *B. diffusa* and *T. cordifolia* provided only moderate prevention against depletion of the enzyme activity (p<0.01). *E. jambolana* did not provide any significant defense to catalase action in retina (p>0.05).

13. Histopathological findings confirmed that the experimental drugs (some higher and some to lower extent) were able to prevent degenerative changes in retinal vasculature, thus providing protection against diabetic retinopathy. PHC proved to
be best effective drug and *E. jambolana* the least in management of diabetic retinopathy

14. PHC showed best preventive effects followed by MC > BD > TC > EJ
7.2. Conclusions

- *Momordica charantia*, *Boerhavia diffusa*, *Eugenia jambolana* and *Tinospora cordifolia* extracts significantly prevented the abnormal retinal changes in diabetic retinopathy possibly due to their anti-hyperglycemic, antioxidant, anti-angiogenic and anti-inflammatory properties in both type-1 and type-2 models of diabetic retinopathy.

- Histopathology supported the effects shown by the treatment with the extracts.

- It is concluded that all the treatments studied are capable of preventing the changes of diabetic retinopathy.

- Co-administration of the drugs as Polyherbal combination (PHC) has shown synergistic action and exhibited most pronounced effects than that of individual drugs. PHC thus, possesses potential of being used for the prevention of diabetic retinopathy due to type-1 or type-2 diabetes.

- Among individual drugs *M. charantia* proved to be most effective followed by *B. diffusa* and *T. cordifolia* respectively. Efficacy of *E. jambolana* was least as compared to other three drugs. It can thus be used as an adjuvant drug along with other therapeutically active drugs.

- Herbal extracts used in the study were able to prevent the changes associated with diabetic retinopathy in rats and can be explored clinically.

- Beneficial effects of the other drugs can be explored for their therapeutic potential.

- More studies involving active constituents of herbal drugs are underway and will provide insights towards the mechanisms of action of the herbal extracts.
FUTURE IMPLICATIONS

Keeping in view the encouraging results with the extracts of *Momordica charantia*, *Boerhavia diffusa*, *Eugenia jambolana* and *Tinospora cordifolia* and their combination (PHC), it is suggested that following studies be conducted in order to establish their mechanism of action:

- Active constituents present in the extracts, which are responsible for the effects can be identified and isolated.

- In-vitro studies involving gene expression should be conducted so that exact molecular mechanism can be established.