5.2. Type-II Diabetic Retinopathy

5.2.1. *Momordica charantia*

5.2.1.1. Role of *M. charantia* in management of Glycemic Parameters in Type-2 Diabetic Rats

A. Blood glucose

The animals that developed diabetes after 6 weeks of STZ injection were treated with *M. charantia* (MC) extract. Untreated control group had significant increase in blood sugar level as compared to normal animals. The antihyperglycemic effect of *M. charantia* was studied in STZ treated Wistar rats. 24 week treatment with *M. charantia* resulted in remarkable glycemic control as recorded on weekly estimation of blood sugar showing significant decrease (p<0.001) as compared with STZ treated controls (Figure 5.2.1)

![Blood Glucose Graph](image)

**Figure 5.2.1.** Effect of *M. charantia* on blood glucose in T-2 DR. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).**
B. Glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin (HbA1c) is considered as a more reliable index of glycemic control than fasting blood values. We therefore estimated HbA1c values of normal, diabetic control and treated groups after 24 weeks of treatment. HbA1c in the non-diabetic group was 4.52 ± 0.29%, while it was significantly more (p<0.001) in diabetic control group (7.04 ± 0.51%). However after treatment with *M. charantia* extract, a significant reduction in HbA1c was observed (5.45 ± 0.72%, p<0.001) when compared with diabetic control value (Figure 5.2.2).

![Figure 5.2.2. Effect of *M. charantia* on %HbA1c. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).](image)

5.2.1.2. Effect of *M. charantia* on body weight and food and water intake of animals

The gain in body weight in the untreated diabetic rats was much less than that in normal rats. But after treatment for 24 weeks with extract of *M. charantia*, a significant gain in body weight (p<0.01) in the treated rats indicated reversal of diabetes This showed MC to have a long lasting effect in the experimental diabetic condition, which may be due to its action at the tissue or receptor level (Table 5.11).
Daily consumption of water and food in healthy adult rats were measured as 22.59 ± 3.15 ml and 12.05 ± 1.13 grams respectively. But in diabetic rats, daily consumption of water and food were measured as 52.03 ± 2.51 ml and 26.91 ± 2.33 grams (Table 5.1). However, diabetic animals treated with *M. charantia* showed significant increase in water (33.13±1.92 ml) and food intake (14.89±0.87) as compared to untreated diabetic rats (p<0.0001)

**Table 5.11 Effects of the oral administration of *M. charantia* extract on the body weight and food and water consumption of STZ-induced type II diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th><em>M. charantia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>% change in body weight</td>
<td>57.54±4.69*</td>
<td>37.34±5.92</td>
<td>51.07±3.40*</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>22.59±3.15*</td>
<td>52.03±2.51</td>
<td>33.13±1.92*</td>
</tr>
<tr>
<td>Food intake (gm)</td>
<td>12.05±1.13*</td>
<td>26.91±2.33</td>
<td>14.89±0.87*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control

5.2.1.3. Ocular examination

A. Effect of *M. charantia* on Anterior Segment Changes

Photographs of lens were taken using slit lamp microscope. Normal control rats’ lenses represented a clear picture throughout the study period. Lens of diabetic control rats were normal in the early weeks of study, but slowly progressed to the development of cataract. By the end of 24 weeks, cataractous changes appeared at the centre of the lens as shown by arrow in the figure 5.2.3 (a-c). Photographs were evaluated for cataract score. Non-diabetic animals were scored zero. Average cataract score for diabetic lenses was 17. However, rats treated with *M. charantia* showed only mild changes as compared to diabetic control group with average cataract score 04 (Table 5.12).
B. Effect of M. charantia on Posterior Segment Changes

Retinal photographs taken by fundus camera revealed a distinct and clear structure of fundus in healthy rats. But in case of diabetic control group, fundus pictures appeared to be dull and hazy, which might be due to originating opacity as a result of hyperglycemia. Their retinal vessels got dilated as compared to normal animals. Treatment with M. charantia protected the development of any opacity or tortuosity in diabetic rats and distinct fundus was visible, with significantly lesser diameter as compared to untreated diabetic group (Figure 5.2.3 d-f; & Table 5.12).

Figure 5.2.3. Photographs showing lenticular and retinal changes (a) Normal rat lens (b) Diabetic lens showing cataract (shown by arrow) (c) M. charantia treated lens showing no abnormality (d) Normal rat fundus (e) Diabetic rat fundus with mild tortuosity (f) M. charantia treated rat fundus
Table 5.12 Effects of *M. charantia* extract on cataract score and vessel diameter of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th><em>M. charantia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cataract score</strong></td>
<td>0*</td>
<td>17</td>
<td>4*</td>
</tr>
<tr>
<td><strong>Vessel diameter (Pixels)</strong></td>
<td>16.54±1.76*</td>
<td>27.23±1.65</td>
<td>20.03±1.25*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control

C. Fluorescein angiography

Fluorescein angiogram of normal animal represented smooth circulation of fluorescein dye into each vein of retina. No leakage or blockage was observed thus, indicating proper blood flow. In diabetic control rat, the fundus was not clearly visible due to cataractous changes appearing in anterior segment. So appearance of any microaneurysm or ischemia could not be confirmed. In *M. charantia* treated rat, dye appearing in the vessels was clearly visible, indicating absence of ischemia or hypoxia (figure 5.2.4).

Figure 5.2.4. Fluorescein angiogram of retina of study groups at the end of 24 weeks of treatment.
NC: Normal control; DC: Diabetic control; MC: *M. charantia* treated
5.2.1.4. Effect of *M. charantia* on Angiogenic parameters

**A. Vascular Endothelial Growth Factor (VEGF)**

The retinal VEGF level of normal control, diabetic control and *M. charantia* treatment groups determined by ELISA is represented in figure 5.2.5. The retinal VEGF concentration was higher in untreated diabetic control group than in the non-diabetic (normal control) group (p<0.001). This upregulation was significantly prevented/attenuated by *M. charantia* (p<0.001), where VEGF level reduced by 55% as compared to diabetic control group.

![Figure 5.2.5](image-url)  
*Figure 5.2.5. Effect of *M. charantia* on angiogenic marker VEGF. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).*

**B. Protein Kinase-C (PKC)**

Figure 5.2.6 shows the concentration of PKC in retina of study animals. Retinal PKC level were significantly higher in diabetic control than normal group by the end of study (p<0.001). In *M. charantia* treated diabetic rats, PKC had decreased significantly in a span of 24 weeks (p<0.001), indicating the efficacy of the drug.
Figure 5.2.6. Effect of *M. charantia* on angiogenic marker PKC. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.1.5. Anti-inflammatory Actions of *M. charantia* in Type-2 Diabetic Retinopathy

**A. Tumor Necrosis Factor (TNF-α)**

In the retina obtained from the untreated rats diabetic for 24 weeks, TNF-α levels remained 2.5 times elevated as compared with those obtained from age-matched normal rat retina (p<0.001) (Figure 5.2.7). Diabetes induced changes in retinal TNF-α expression was significantly inhibited by oral administration of *M. charantia* (p<0.01), which resulted in 1.8 times decrease of cytokine level in the treatment group.
RESULTS - Type 2 DR

Figure 5.2.7. Effect of *M. charantia* on TNF-α level in Type-2 DR. Each value represents mean ± S.D (n=6). ***p<0.001 and **p<0.01 vs. DC. One Way ANOVA (Student-Newman-Keuls Method).

B. Interleukin-1beta (IL-1β)

The concentration of the inflammatory cytokine IL-1β was elevated by over 3.2 folds in the retina of rats diabetic for 24 weeks as compared with those in the retina obtained from age-matched normal control rats (Figure 5.2.8). Long-term administration of MC resulted in significant inhibition of diabetes-induced increase in retinal IL-1β level in the treatment group (p<0.001).

Figure 5.2.8. Effect of *M. charantia* on inflammatory parameter IL-1β. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
5.2.1.6. Effect of *M. charantia* on antioxidant enzymes in type-2 diabetic retinopathy

A. Glutathione (GSH)

Twenty four weeks of diabetes decreased antioxidant capacity, glutathione levels in the retina by more than half compared to the values obtained from normal control rats (p<0.001). *M. charantia* supplementation in diabetic rats prevented decrease in retinal GSH levels. The values in the *M. charantia*-treated diabetic rats were significantly higher (p<0.001) compared to untreated (diabetic control) group (Figure 5.2.9).

![Figure 5.2.9. Effect of *M. charantia* on antioxidant glutathione (GSH) concentration. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).](image)

B. Catalase

Oxidative stress was determined by evaluating concentration of antioxidant enzyme catalase. The antioxidant capacity of retinal catalase level was decreased by about 2.3 folds in diabetes compared to the age-matched normal control rats (p<0.001). Administration of *M. charantia* provided protection against diabetes induced depletion of enzyme level in retina. The amelioration due to *M. charantia* in treated animals was statistically different (p<0.001) from diabetic control values (Figure 5.2.10).
RESULTS - Type 2 DR

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>8.60</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>3.75</td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>6.46</td>
</tr>
</tbody>
</table>

Figure 5.2.10. Effect of *M. charantia* on catalase enzyme activity. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.1.7. Histological analysis of retina

Retinae of the normal control, diabetic control and treatment groups were subjected to histopathological changes in order to evaluate the abnormal increase in thickness of retinal basement membrane during the course of our study. Evaluation was done by semi-quantitative grading method (Annexure 3-B), where depending upon the severity, abnormal changes were classified as Nil (- no significant vessel dilation); mild (+), moderate (++) and severe (+++, maximum dilation).

Blood vessels of non-diabetic animal did not show any abnormal changes and were found to possess thin and long endothelial cells. Retina of diabetic control rats showed maximum dilation of the blood vessels (+++; Annexure 3-B). However, retina of *M. charantia* treated group exhibited a mild thickening (+) of retinal vessel basement membrane (Figure 5.2.11).
Figure 5.2.11. High power photomicrographs of rats’ retinae (HE x 400) showing blood vessels (Ret BV). (a) Normal control retina showing thin and long endothelial cells (b) Diabetic control retina showing dilated blood vessel (c) M. charantia treated retina showed only mild dilation
5.2.2. *Boerhavia diffusa*

5.2.2.1 Role of *Boerhavia diffusa* in management of Glycemic parameters in type-2 diabetic rats

A. Blood glucose

Blood glucose levels in the three study groups are represented in figure 5.2.12. Fasting blood glucose in normal animals was 95.88 ± 4.43 mg/dl. Diabetic control rats possessed significantly higher (174.11 ± 12.06 mg/dl, p<0.0001) blood sugar. However, treatment with *Boerhavia diffusa* (BD) significantly reduced blood glucose level to (145.58 ± 13.97 mg/dl, p<0.001).

![Blood Glucose Graph](image)

Figure 5.2.12. Effect of *Boerhavia diffusa* on blood glucose in T-2 DR. Each value represents mean ± S.D (n=6). **p<0.0001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Glycated hemoglobin (HbA1c)

The %HbA1c levels were measured at the end of 24th week of treatment. In normal group glycosylated hemoglobin was 4.52 ± 0.29% while the same was found to be 7.04 ± 0.51% in diabetic control group, which was significantly lower than the normal animals (p<0.001). In *B. diffusa* treated group the %HbA1C level reduced significantly to 5.58 ± 0.70 in comparison to untreated diabetic group (Figure 5.2.13).
**Figure 5.2.13.** Effect of *Boerhavia diffusa* %HbA1c. Each value represents mean ± S.D (n=6).

**p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).**

### 5.2.2.2 Effect of *Boerhavia diffusa* on body weight and food and water intake of animals

After six weeks of induction of STZ to 2 days old pups, no significant difference in body weight was observed between normal rats, diabetic control and treatment groups. However by the end of the 24 weeks, weight gain was significantly higher in the normal rats (p<0.01). The percent change in body weight was 57.54 ± 4.69, 37.34 ± 5.92 and 45.76 ± 3.53 for normal, diabetic control and BD treated groups respectively (Table 5.13). Average final weight of treatment group as compared to diabetic control group rats were statistically different (p<0.01).

Animals, which received STZ also showed an increase in water and food intake as compared to normal control, which was significantly reversed by administration of *Boerhaavia diffusa* in 24 weeks of treatment (Table 5.13).
Table 5.13 Effects of the oral administration of *Boerhavia diffusa* extract on the body weight and food and water consumption of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Boerhavia diffusa</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change in body weight</td>
<td>57.54±4.69*</td>
<td>37.34±5.92</td>
<td>45.76±3.53*</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>22.59±3.15*</td>
<td>52.03±2.51</td>
<td>37.38±2.24*</td>
</tr>
<tr>
<td>Food intake (gm)</td>
<td>12.05±1.13*</td>
<td>26.91±2.33</td>
<td>18.68±1.22*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control

5.2.2.3 Ocular examination

A. Effect of *Boerhavia diffusa* on Anterior segment changes

Photographs of anterior segment of eyes of normal control, diabetic control and *Boerhavia diffusa* (BD) treated groups are represented in figure 5.2.14 (a-c). Eyes from diabetic control group showed signs and symptoms of cataract development (shown by arrow) when anterior chamber photographs were evaluated. However, cataractous changes were absent in normal and treatment groups Score of cataract development in diabetic control animals was 17 which was significantly higher (p<0.0001) than BD treated group having score 6 at the end of 24th week (Table 5.14).

B. Effect of *Boerhavia diffusa* on Posterior segment changes

As observed in retinal photographs by fundus camera, tortuosity and opacity gradually developed in diabetic control (DC) group in a span of 24 weeks but was absent in the normal group (NC). Distinct significant thickening of vessel diameter was present in the diabetic group (p<0.0001). However fundus changes were not prominent in the BD treated group and vessel diameter in the treatment group was significantly lesser than untreated diabetic group (Figure 5.2.14 d-f; Table 5.14).
Figure 5.2.14. Photographs showing lenticular and retinal changes at the end of the study (a) Normal rat lens (b) Diabetic lens with cataract (indicated by arrow) (c) *Boerhavia diffusa* treated lens (d) Normal rat fundus (e) Haziness appearing in Diabetic rat fundus (f) *Boerhavia diffusa* treated rat fundus

Table 5.14 Effects of the oral administration of *Boerhavia diffusa* extract on cataract score and vessel diameter of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th><em>Boerhavia diffusa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of clock hours</td>
<td>0**</td>
<td>17</td>
<td>6**</td>
</tr>
<tr>
<td>affected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>16.54±1.76**</td>
<td>27.23±1.65</td>
<td>22.57±1.39*</td>
</tr>
<tr>
<td>(Pixels)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). **p<0.0001; *p<0.001 vs Diabetic control
C. Fluorescein angiography

A clear pattern of retinal vessels was depicted in the angiogram of non-diabetic animal. In case of diabetic control retina, traces of fluorescein dye could be seen apart from vessels too. This leakage might be the reason of improper blood circulation due to hypoxia or ischemia. BD treated group did not reveal any leakage of dye outside the retinal vessels (Figure 5.2.15).

![Fluorescein angiogram of Normal control (NC), Diabetic control (DC) and B. Diffusa (BD) treated rats after 24 weeks of treatment](image)

Figure 5.2.15. Fluorescein angiogram of Normal control (NC), Diabetic control (DC) and B. Diffusa (BD) treated rats after 24 weeks of treatment

5.2.2.4 Effect of Boerhavia diffusa on Angiogenic parameters

A. Vascular Endothelial Growth Factor (VEGF)

Retinal level of VEGF in non-diabetic animals was 5.45±1.65 pg/mg protein. A significant rise (p<0.001) was observed in the expression of VEGF levels in diabetic control group (15.30±2.26 pg/ mg protein) as compared to normal group. However, B. diffusa was found to significantly protect (p<0.001) the rise in the expression of VEGF levels (8.23±1.32 pg/ mg protein) in treatment group as compared to untreated diabetic group (Figure 5.2.16).
Figure 5.2.16. Effect of *Boerhavia diffusa* on VEGF. Each value represents mean ± S.D (n=6).

**p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Protein kinase-C (PKC)

Angiogenic factor PKC was 59.09 ± 24.03 ng/mg protein in normal group retina. Untreated diabetic animals showed high increase in PKC level (117.59 ± 11.61 ng/mg protein; p<0.001). However, on administration of *B. diffusa* for 24 weeks, treated animals showed significant decrease in retinal PKC level (83.37 ± 22.16, p<0.001) as compared to diabetic control group (Figure 5.2.17).
5.2.2.5 Anti-inflammatory actions of *Boerhavia diffusa* in type-2 diabetic retinopathy

**A. Tumor Necrosis Factor (TNF-α)**

A significant rise (p<0.001) was observed in the expression of TNF-α level in diabetic control group (65.79 ± 20.65 pg/mg protein) as compared to normal group (26.09 ± 17.13 pg/mg protein). However, *B. diffusa* was found to protect the rise significantly (p<0.05) in the expression of TNF-α levels (48.17 ± 26.80 pg/mg protein) in the treatment group when compared with control group (Figure 5.2.18).
RESULTS - Type 2 DR

Figure 5.2.18. Effect of *Boerhavia diffusa* on inflammatory marker TNF-α. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.05 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

**B. Interleukin-1beta (IL-1β)**

IL-1β in normal animals was 39.40 ± 20.64 pg/mg protein. Diabetic animals showed a highly significant increase (127.82 ± 15.12 pg/mg protein, p<0.001) in expression of cytokine level in retina. 24 week treatment with *B. diffusa* (BD) reduced IL-1β expression to 68.99 ± 27.37 pg/mg protein, which was significantly different from that of diabetic control group (p<0.001) (Figure 5.2.19).
Figure 5.2.19. Effect of BD on inflammatory parameter IL-1β. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.2.6 Effect of Boerhavia diffusa on antioxidant enzymes in type 2 diabetic retinopathy

A. Reduced Glutathione (GSH)

A significant fall (p<0.001) was observed in GSH levels in diabetic control group (4.28±1.03 nmol/mg protein) as compared to normal group (9.04±1.92 nmol/mg protein). However, *B. diffusa* was found to protect depletion of GSH levels significantly (6.77±1.86 nmol/mg protein) in the treatment group as compared to diabetic control animals (p<0.001) (Figure 5.2.20).
RESULTS - Type 2 DR

Figure 5.2.20. Effect of Boerhavia diffusa on GSH concentration. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Catalase

The activity of enzyme catalase reduced significantly (p<0.001) in diabetic control group (3.75 ± 1.39 IU/mg protein) in comparison to that of the normal group (8.60 ± 2.80 IU/mg protein). However, treatment with B. diffusa preserved the catalase activity and restored the retinal enzyme expression to 6.01 ± 1.86 IU/mg protein (p<0.01) (Figure 5.2.21).

Figure 5.2.21. Effect of Boerhavia diffusa on Catalase activity. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.01 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
5.2.2.7 Retinal Histology

Histopathological evaluation of retinae of study groups’ animals is represented in figure 5.2.22. Diabetic control rat retina represented a gross increase in membrane thickness (+++) in comparison to normal rat retina (--). However, retinal basement membrane of *B. diffusa* treated retina showed a little increase (+) as compared to diabetic control group (Annexure-3B).

Figure 5.2.22. High power photomicrographs (HE x 400) showing blood vessel (Ret BV) from the retina of study groups. (a) Non-diabetic group represented normal vasculature (b) Diabetic control group showed increased thickness of vascular basement membrane (c) *B. diffusa* treated retina showed partial increase in membrane thickness
**5.2.3. Eugenia jambolana**

5.2.3.1 Role of *Eugenia jambolana* in management of Glycemic parameters in type-2 diabetic rats

**A. Blood glucose**

Blood glucose in diabetic control animals showed a highly significant increase when compared to normal non-diabetic animals (p<0.0001). Administration of *Eugenia jambolana* (EJ) for 24 weeks reduced the glucose level to 153.46 ± 14.52 mg/dl, which was statistically lesser (p<0.001) than untreated diabetic group (Figure 5.2.23).

![Blood Glucose Graph](image)

Figure 5.2.23. Effect of *Eugenia jambolana* on blood glucose in type-2 diabetic rats. Each value represents mean ± S.D (n=6), ***p<0.0001; **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

**B. Glycated hemoglobin (HbA1c)**

HbA1c measured after 24 weeks of diabetes was found to be higher (p<0.001) in the diabetic control animals as compared to that of normal animals. However, diabetic rats treated with *E. jambolana* showed significant reduction (p<0.01) in HbA1c values as compared to diabetic control animals (Figure 5.2.24).
5.2.3.2 Effect on body weight and food and water intake

At the end of 24 weeks of treatment, average body weight of the animals of three groups differed as shown in the table 5.15. There was a significant difference (p<0.0001) in the percent weight change between normal and the diabetic control groups (table 5.15). However, the difference between the percent change in body weights of diabetic and EJ treated group was not significant (p>0.5).

Similarly, food and water intake in non diabetic animals was significantly lesser when compared to other groups. On the other hand, consumption among treatment and diabetic groups showed no significant difference (table 5.15).
Table 5.15 Effects of the oral administration of *Eugenia jambolana* extract on the body weight and food and water consumption of STZ-induced type II diabetic rats (*p<0.0001 vs DC*)

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th><em>Eugenia jambolana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>% change in body weight</td>
<td>57.54±4.69*</td>
<td>37.34±5.92</td>
<td>41.32±2.65</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>22.59±3.15*</td>
<td>52.03±2.51</td>
<td>49.38±3.24*</td>
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<tr>
<td>Food intake (gm)</td>
<td>12.05±1.13*</td>
<td>26.91±2.33</td>
<td>21.62±1.50*</td>
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</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control

5.2.3.3 Ocular examination

**A. Effect of *Eugenia jambolana* on Anterior segment changes**

Development of cataractous changes could be seen in diabetic control group on evaluation of the lens photographs taken by slit lamp. On the other hand, anterior segment of non-diabetic animals showed normal picture with no sign for cataract. However, little abnormal changes were seen in anterior chambers of animals treated with *E. jambolana* (Figure 5.2.25 a-c). Score for cataract of the three groups are presented in table 6.6.

**B. Effect of *Eugenia jambolana* on Posterior segment changes**

**Fundus changes**

Retinal photographs taken from fundus camera were evaluated for the posterior segment changes in the study groups and vessel diameter was measured. Non-diabetic group showed normal radial vasculature with vessel diameter 16.54±1.76 pixels. Significant thickening of vessel occurred in the diabetic group as compared to normal non-diabetic animals. However, there was no significant difference in the vessel diameters of diabetic control and EJ treated groups (Figure 5.2.25 d-f; table 5.16).
Figure 5.2.25. Photographs showing lenticular and retinal changes at the end of 24 weeks of diabetes (a) Normal rat lens (b) Diabetic lens showing development of cataract (c) Eugenia jambolana treated lens (d) Normal fundus (e) Diabetic dilated fundus (f) Eugenia jambolana treated rat fundus

Table 5.16 Effects of the oral administration of Eugenia jambolana extract on cataract score and vessel diameter of STZ-induced type II diabetic rats (*p<0.0001 vs DC)

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Eugenia jambolana</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cataract score</strong></td>
<td>0*</td>
<td>17</td>
<td>10*</td>
</tr>
<tr>
<td><strong>Vessel diameter (Pixels)</strong></td>
<td>16.54±1.76*</td>
<td>27.23±1.65</td>
<td>25.04±0.73*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control
Fluorescein angiography

Fluorescein angiogram of diabetic control group was not clearly visible under fluorescein fundus camera because of development of opacity. Thus path of fluorescein dye flow could not be traced. However clear vascular architecture was observed in normal and EJ treated rats with no leakage of dye (Figure 5.2.26).

5.2.3.4 Effect of *Eugenia jambolana* on Angiogenic Parameters

**A. Vascular Endothelial Growth Factor (VEGF)**

In the present investigation, retinal VEGF, an important angiogenic marker in diabetic retinopathy, was lower in normal animals but significantly increased by 2.8 times in diabetic control group (p<0.001). However, treatment with EJ for 24 weeks significantly showed about 34% inhibition in the rise in VEGF expression levels (p<0.001) as compared to that of non-treated animals (Figure 5.2.27).
B. Protein Kinase-C (PKC)

Diabetic control group exhibited about 2-folds significant increase in PKC level as compared to non-diabetic group (p<0.001). Although the administration of *E. jambolana* reduced PKC expression in retina of the treatment group by 12%, but the change was not significant (p>0.05) when compared with untreated diabetic animals (Figure 5.2.28).

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**Figure 5.2.27.** Effect of *Eugenia jambolana* on VEGF in type-2 diabetic retinopathy. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

**Figure 5.2.28.** Effect of *Eugenia jambolana* on PKC in type-2 diabetic retinopathy. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
5.2.3.5 Anti-inflammatory actions of *E. jambolana* in type-2 diabetic retinopathy

A. Tumor Necrosis Factor (TNF-α)

TNF-α in diabetic control animals showed a significant marked upregulation and the level reached to 2.5 times higher than that of normal animals (p<0.001). Treatment with *E. jambolana*, reduced the expression of TNF-α in retina, but could not provide a significant protection to the treated animals (p>0.05) as compared to diabetic control animals (Figure 5.2.29).

![Figure 5.2.29. Effect of *Eugenia jambolana* on TNF-α. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).](image)

B. Interleukin-1beta (IL-1β)

In the present investigation IL-1β in normal animals was significantly lower as compared to diabetic control animals (p<0.001). Diabetic group exhibited approximately 3-times higher level of the cytokine than that of normal group. Treatment with *E. jambolana* for 24 weeks significantly reduced (p<0.05) the IL-1β retinal expression by 25.6% when compared with diabetic control group (Figure 5.2.30).
5.2.3.6 Effect of *Eugenia jambolana* on antioxidant enzymes in type-2 diabetic retinopathy

**A. Reduced Glutathione (GSH)**

Retina of non-diabetic animals possessed high antioxidant activity. Significant depletion of glutathione level was observed in diabetic control animals as compared to normal control group (p<0.001). However, oral administration of *E. jambolana* for 24 weeks increased GSH activity significantly (p<0.05) in retina when compared to untreated animals (Figure 5.2.31).
Figure 5.2.31. Effect of *Eugenia jambolana* on GSH concentration. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.05 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Catalase

The activity of catalase significantly reduced by 56% in diabetic control group as compared to normal group (p<0.001). *E. jambolana* was not able to provide significant protection (p>0.05) to the enzyme activity in treatment group in comparison to diabetic control animals (Figure 5.2.32).

Figure 5.2.32. Effect of *Eugenia jambolana* on catalase activity. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
5.2.3.7 Retinal Histology

Histopathology of retina of non diabetic rat showed blood vessel with thin and long endothelial cells. On the other hand diabetic control rat retina showed thicker basement membrane (+++) as compared to normal. Moderate increase was found in retinal membrane thickness of *E. jambolana* treated group (+) but was lesser than that of untreated diabetic group (Figure 5.2.33, Annexure-3B).

![Figure 5.2.33](image_url)

Figure 5.2.33. High power photomicrographs (HE x 400) of retinal blood vessel (Ret BV). (a) Non-diabetic group showed normal vasculature (b) Diabetic control group showed appreciable dilatation of retinal membrane (c) *E. jambolana* treated retina showed a moderate increase in membrane thickness
5.2.4. *Tinospora cordifolia* (TC)

5.2.4.1. Role of TC in management of glycemic parameters in type-2 diabetic rats

A. Blood glucose

Normal group rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ led to about 2 times elevation of blood glucose levels as compared to normal group over a period of 24 weeks. However, as shown in figure 5.2.34, treatment with *T. cordifolia* exhibited a significant reduction in blood glucose by 16% (p<0.001) as compared to that of untreated rats.

![Figure 5.2.34.](image)

**Figure 5.2.34.** Effect of *T. cordifolia* on blood glucose in type-2 DR. Each value represents mean ± S.D (n=6). **p<0.0001; *p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Glycated hemoglobin (HbA1c)

Percent HbA1c was measured in all the groups after 24 weeks of treatment. It was observed that % HbA1c in diabetic control animals was significantly higher than that of normal group (p<0.001). Rats treated with *T. cordifolia* showed reduced HbA1c (p<0.01)
and the difference was statistically significant when compared with diabetic group (Figure 5.2.35).

**Figure 5.2.35.** Effect of TC on % HbA1c. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.01 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.4.2. Effect of TC on body weight and food and water intake of animals

Table 5.17 below illustrates the variations in body weight and food & water intakes of normal control, diabetic control and diabetic treated groups. Induction of STZ significantly reduced the body weight compared with the normal group (p<0.001). Percent change in body weight of treatment group was significantly more than untreated animals (p<0.05).

Diabetic control rats showed significantly higher intake of food and water when compared with normal control groups (p<0.0001). The food and water consumption, as shown in table 5.17, was significantly decreased in diabetic rats treated with *T. cordifolia* (p<0.0001).
Table 5.17 Effects of the oral administration of TC extract on the body weight and food and water consumption of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Tinospora cordifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change in body weight</td>
<td>57.54±4.69**</td>
<td>37.34±5.92</td>
<td>45.57±6.84*</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>22.59±3.15**</td>
<td>52.03±2.51</td>
<td>40.79±1.94**</td>
</tr>
<tr>
<td>Food intake (gm)</td>
<td>12.05±1.13**</td>
<td>26.91±2.33</td>
<td>19.99±1.07**</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). **p<0.0001; *p<0.01 vs Diabetic control

5.2.4.3. Ocular examination

A. Effect of TC on Anterior segment changes

Abnormal lenticular changes were observed in diabetic eyes of animals, however normal eyes showed clear architecture of lenses (Figure 5.2.36 a-c). Anterior segment photos of T. cordifolia treated animals did not reveal much change and when scored for cataract, it was significantly lower than that of diabetic group (Table 5.18).

B. Effect of TC on Posterior segment changes

Fundus changes

Figure 5.2.36 (d-f) shows the retinal photographs of animals at the end of 24 weeks. Healthy normal animals did not show any haziness or other signs of cataract, but haziness developed in fundus of diabetic animal with significant thickening of vessel diameter (p<0.0001). The retinal photographs of TC treated animal showed only mild changes and exhibited significantly smaller vessel diameter (p<0.05) as compared to diabetic control group (Table 5.18).
RESULTS - Type 2 DR

Figure 5.2.36. Photographs showing lenticular and retinal changes after 24 weeks of treatment (a) Normal rat lens (b) Diabetic lens with cataract (c) TC treated lens (d) Normal fundus (e) Diabetic rat dilated fundus (f) TC treated rat fundus

Table 5. 18 Effects of the oral administration of TC extract on cataract score and vessel diameter of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Tinospora cordifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract score</td>
<td>0**</td>
<td>17</td>
<td>7**</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>16.54±1.76**</td>
<td>27.23±1.65</td>
<td>24.52±2.26*</td>
</tr>
<tr>
<td></td>
<td>(Pixels)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). **p<0.0001; *p<0.05 vs Diabetic control
Fluorescein angiography

Figure 5.2.37 represents the fluorescein angiogram of normal, diabetic control and TC treated animals. Much of the changes could not be revealed between the three groups except that circulation time of fluorescein dye was maximum in diabetic control rat which might be due to dilation of the blood vessels.

Figure 5.2.37. Fluorescein angiogram of retina of the study groups. NC: Normal control; DC: Diabetic control and TC: T. cordifolia treated

5.2.4.4. Effect of TC on Angiogenic parameters

A. Vascular Endothelial Growth Factor (VEGF)

Significant increase by 2.8 times in VEGF was observed in diabetic retina as compared to non-diabetic one (p<0.001). After 24 weeks of treatment with T. cordifolia expression of the growth factor showed 46% significant reduction (p<0.001) in retinal VEGF when compared with that of untreated group (Figure 5.2.38).
Figure 5.2.38. Effect of *T. cordifolia* on VEGF level. Each value represents mean ± S.D (n=6). **p<0.001 vs DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

**B. Protein kinase-C (PKC)**

Figure 5.2.39 represents retinal PKC levels in normal, diabetic control and treatment groups at the end of the study. A steep rise in retinal expression of PKC occurred in diabetic control group as compared to normal group (p<0.001). However, *T. cordifolia* administration in the treatment group significantly reduced the PKC expression by more than 28% in a span of 24 weeks (p<0.01) as compared to the diabetic group.
5.2.4.5. Anti-inflammatory actions of TC in type-2 diabetic retinopathy

**A. Tumor Necrosis Factor (TNF-α)**

A significant 2.5 times increase in TNF-α expression was observed in diabetic control animals as compared to normal animals (p<0.001). TC administration although reduced the retinal TNF level by 16% in the treatment group, but the change was not statistically significant (p>0.05) when compared with diabetic control group (Figure 5.2.40).
Figure 5.2.40. Effect of *T. cordifolia* on TNF-α. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

**B. Interleukin-1beta (IL-1β)**

Diabetic group showed a steep rise in retinal IL-1β level as compared to the normal group (p<0.001). However, on long term administration of *T. cordifolia*, the cytokine expression significantly reduced by 40% (p<0.001) in retina of the animals in the treatment group (Figure 5.2.41).
5.2.4.6. Effect of *T. cordifolia* on Anti-oxidant Parameters

A. Glutathione

Retina of non-diabetic animals possessed good antioxidant glutathione (GSH) enzyme activity. However, significant depletion of the enzyme was observed in diabetic control animals (p<0.001) as compared to normal controls. Treatment with *T. cordifolia* was however able to restore the GSH activity by 51% in treatment group, which was significantly higher (p<0.01) than that in the untreated diabetic group (Figure 5.2.42).
**RESULTS - Type 2 DR**

![Graph showing GSH levels in Normal Control, Diabetic Control, and Tinospora cordifolia groups.](image)

**Figure 5.2.42.** Effect of *T. cordifolia* on glutathione (GSH) activity. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.01 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).**

**B. Catalase**

Level of retinal catalase activity significantly reduced in diabetic control group in comparison to non-diabetic animals (p<0.001). On administration of *T. cordifolia* the catalase enzyme expression increased in retina in a span of 24 weeks of treatment and the change was significant (p<0.01) when compared with untreated diabetic animals (Figure 5.2.43).
Figure 5.2.43. Effect of *T. cordifolia* on catalase activity. Each value represents mean ± S.D (n=6). **p<0.001 and *p<0.01 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.4.7. Retinal Histology

Blood vessels of non-diabetic animal were found to possess thin and long endothelial cells. Comparatively thicker basement membrane was observed in diabetic control rat (+++). Light microscopy of retina of *T. cordifolia* treated group showed mild dilatation (+) of retinal blood vessel basement membrane as compared to diabetic control group (Figure 5.2.44, Annexure-3B).
Figure 5.2.44. High power photomicrographs of retina (HE x 400) showing retinal blood vessel (Ret BV) in different groups. (a) Normal control retina (b) Diabetic control retina with dilated basement membrane (c) *T. cordifolia* treated retina with mild increase in thickness
5.2.5. *Polyherbal combination (PHC)*

5.2.5.1. Role of PHC in Management of Glycemic Parameters in Type-2 Diabetic Rats

A. **Blood glucose**

Figure 5.2.45 shows the average blood glucose levels of the study groups. It was observed that the fasting blood glucose reached the highest level in the diabetic control group. The animals treated with the poly-herbal extract retained blood glucose levels comparatively lower to that of diabetic animals (p<0.0001).

![Blood Glucose Graph](image)

**Figure 5.2.45.** Effect of PHC on blood glucose in type-2 diabetic rats. Each value represents mean ± S.D (n=6). **p<0.0001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).**

B. **Glycated Hemoglobin (HbA1c)**

Diabetic control animals showed significantly higher glycated hemoglobin than normal animals. However, the rats administered with polyherbal combination showed significantly reduced hyperglycemia (p<0.001) as compared to diabetic group, and restored HbA1c level to 5.22 ± 0.72 % (Figure 5.2.46).
5.2.5.2. Effect on body weight and food and water intake of animals

STZ induced diabetic rats showed a significant decrease in body weight (p<0.0001) compared to normal rats in a span of 24 weeks. Statistically significant increase in body weight was observed in the animals treated with polyherbal extract (p<0.001). Treatment with PHC restored the body weight near to normal values (table 5.19).

A significant increase in food and water intake was observed in diabetic rats compared with the normal control group of rats. However, increase in food and water consumption due to hyperglycemia was not altered in diabetic rats upon feeding with polyherbal extract (p<0.0001; table 5.19).

Figure 5.2.46. Effect of PHC on %HbA1c. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
Table 5.19 Effects of the oral administration of PHC extract on the body weight and food and water consumption of STZ-induced type II diabetic rats (*p<0.001 vs DC)

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Polyherbal combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change in BW (%)</strong></td>
<td>57.54±4.69*</td>
<td>37.34±5.92</td>
<td>53.91±5.01*</td>
</tr>
<tr>
<td><strong>Water intake (ml)</strong></td>
<td>22.59±3.15*</td>
<td>52.03±2.51</td>
<td>29.77±3.30*</td>
</tr>
<tr>
<td><strong>Food intake (gm)</strong></td>
<td>12.05±1.13*</td>
<td>26.91±2.33</td>
<td>16.59±1.02*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.001 vs Diabetic control

5.2.5.3. Ocular examination

A. Effect of PHC on anterior segment changes

Lens of non-diabetic group showed a clear structure while diabetic rats exhibited a strikingly increased incidence of cataract invading the nuclear part of the lens as represented by arrow (Figure 5.2.47 a-c). Diabetic control group scored highest for cataract. However, protection with PHC extract inhibited the cataractogenesis in the treatment group. Cataract score for the treatment group was significantly lesser (p<0.0001) than the diabetic control group (table 5.20).

B. Effect of PHC on posterior segment changes

Retinal photographs taken by fundus camera are presented in figure 5.2.47 (d-f). Normal pattern of fundus was observed in the animals of non-diabetic group. Retina of diabetic control group appeared hazy and showed significantly dilated vessels (p<0.0001) as compared to normal group. However, no tortuosity or retinal dilation appeared in the fundus photos of animals treated with PHC. Vessel diameter of the treated group was significantly lower than that of diabetic controls (Table 5.20).
Results

Type 2 DR

Figure 5.2.47. Photographs showing lenticular and retinal changes at the end of 24 weeks of treatment (a) Normal rat lens (b) Diabetic lens with cataract (c) PHC treated lens (d) Normal fundus (e) Haziness appearing in diabetic rat fundus (f) PHC treated rat fundus

Table 5. 20 Effects of the oral administration of PHC extract on cataract score and retinal vessel diameter of STZ-induced type II diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Polyherbal combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract score</td>
<td>0*</td>
<td>17</td>
<td>2*</td>
</tr>
<tr>
<td>Vessel diameter (Pixels)</td>
<td>16.54±1.76*</td>
<td>27.23±1.65</td>
<td>18.31±1.49*</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D (n=6). *p<0.0001 vs Diabetic control

Fluorescein angiography

The fluorescein angiograms in figure 5.2.48 show a clear pattern of retinal vessels in normal control and PHC treated animals. No leakage of dye was seen. However, due to
development of cataract in untreated diabetic animal, clear picture was not observed and path of dye circulation could not be traced.

Figure 5.2.48. Fluorescein angiogram of rats at the end of 24 weeks (a) NC: Normal control rat (b) DC: Diabetic control rat (c) PHC: Polyherbal extract treated rat

5.2.5.4. Effect of PHC on Angiogenic Parameters

A. Vascular Endothelial Growth Factor (VEGF)

There was a significant increase in VEGF in retina of diabetic rats compared with normal rats (p<0.001). Interestingly, PHC extract significantly inhibited the formation of VEGF by 64.7% in the tissue and the level of this growth factor was significantly higher (p<0.001) as compared to diabetic control animals (Figure 5.2.49).
B. Protein kinase-C (PKC)

A steep rise in PKC level occurred in diabetic control group (p<0.001) as compared to normal control in a period of 24 weeks. However, treatment with polyherbal combination significantly reduced the PKC expression (p<0.001) in the retina of treated animals as compared to those of untreated, and restored the values to normal level (Figure 5.2.50).
Figure 5.2.50. Effect of PHC on angiogenic marker PKC. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.5.5. Anti-inflammatory actions of PHC in type-2 diabetic retinopathy

A. Tumor Necrosis Factor (TNF-α)

Effect of polyherbal extract on anti-inflammatory actions are expressed in figure 5.2.51 TNF-α level was significantly higher in retina of untreated diabetic animals when compared with non-diabetic animals (p<0.001). Treatment with PHC to the diabetic animals significantly prevented the increase of retinal TNF-α as compared to that in untreated animals (p<0.001).
RESULTS - Type 2 DR

Figure 5.2.51. Effect of PHC on TNF-α. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

B. Interleukin-1beta (IL-1β)

Retinal IL-1β expression was increased in diabetic group animals as compared to normal control group (p<0.001; Figure 5.2.52). Polyherbal extract was able to prevent the increase in interleukin activity in the retina of treated animals. Level of IL-1β in the treatment group was statistically different than that in diabetic control group (p<0.001).

Figure 5.2.52. Effect of PHC on IL-1β. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).
5.2.5.6. Effect of PHC on antioxidant enzymes in type-2 diabetic retinopathy

A. Reduced Glutathione (GSH)

The activity of the antioxidant glutathione is summarized in figure 5.2.53. Glutathione (GSH) activity was significantly lower in the retina of diabetic animals compared with normal animals (p<0.001). 24 week treatment with PHC was able to restore the activity of retinal GSH in the treatment group significantly as compared to untreated diabetic control group (p<0.001).

![Figure 5.2.53. Effect of PHC on glutathione (GSH) activity. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).](image)

B. Catalase

Catalase activity was found to be altered in the diabetic rat tissue compared with the normal retina (Figure 5.2.54). However, PHC was effective in reversing these changes and showed activity of catalase elevated by 73% in the retina of treated diabetic animals which was significantly higher from that of diabetic control animals (p<0.01).
Figure 5.2.54. Effect of PHC on catalase enzyme activity. Each value represents mean ± S.D (n=6). **p<0.001 vs. DC. One Way Analysis of Variance (Student-Newman-Keuls Method).

5.2.5.7. Histological analysis

Retina of non-diabetic rats and exhibited normal arrangement of retinal blood vessel with presence of thin, long endothelial cells. Whereas, in diabetic control group thickness was maximum and graded as severe (+++). Mild dilation was also observed in PHC treated retina (+) as compared to diabetic control retina (Figure 5.2.55, Annexure-3B).
Figure 5.2.55. High power photomicrographs showing blood vessel (Ret BV) from the retina of rat (a) Normal control retina (b) Significantly dilated diabetic control retina (c) Polyherbal treated retina with mild increase in thickness