RESULTS

a) Biochemical Analysis

TPC of different percentage of EEMC

Figure 27a: Percentage of total phenol content in 50%, 70% and 100% EEMC.
The data obtained showed that, the 50% ethanolic extract had the highest phenol content (0.029%) followed by the 70% (0.0098%) and 100% (0.0022%) ethanolic extracts respectively (Fig 27a) (p=<0.0001) using gallic acid as standard ($R^2=0.9236$). Since a gradient extraction was followed, most of the water soluble fractions of the phenols were found in 50% extracts than ethanol soluble. Further experiments were planned based on the total phenol content in each of these extracts.

TFC in different percentage of EEMC

Figure 27b: Percentage of total flavanoid content in 50%, 70% and 100% EEMC.
Legend: 50% ethanolic extract had the highest flavanoid content (22%) when compared to 70% and 100% ethanolic extract. The inset shows the standard graph with $R^2 = 0.9985$.
The data obtained showed that, the 50% ethanolic extract had the highest flavanoid content (22%) followed by the 70% (14.5%) and 100% (11%) ethanolic extracts respectively (Fig.1) (p=<0.016) using quercetin as standard ($R^2=0.9985$). Since a gradient extraction was followed, most of the water soluble fractions of the flavonoids were found in 50% extracts than 70% and 100% extracts.
Reducing sugar in different percentage of EEMC

![Graph showing percentage of reducing sugar in 50%, 70%, and 100% EEMC.]

Figure 28: Percentage of reducing sugar in 50%, 70% and 100% EEMC. Legend: 50% ethanolic extract had the highest percentage of reducing sugar (2.3%) when compared to 70% and 100% ethanolic extract. The inset shows the standard graph with $R^2 = 0.9920$

The data obtained showed that, the 50% ethanolic extract had the highest percentage of reducing sugar (2.3%) followed by the 70% (0.51%) and 100% (0.06%) ethanolic extracts respectively (Fig 28) ($p<0.0001$) using D-glucose as standard ($R^2=0.9920$).

Identification of Sugar in 50% EEMC by Paper Chromatography

In the paper chromatography the distance moved by the sample corresponded to that of the glucose standard (Fig 29). This was confirmed by calculating the Rf values which is represented in table 1. So the reducing sugar present in the extract may be Glucose.

The presence of glucose was confirmed by GOD-POD method, which showed 332mg/dl of glucose in the 50% EEMC.
Figure 29: Ascending paper chromatography for qualitative estimation of reducing sugars in the 50% extract.

Legend: The distance moved by the sample corresponds to that of glucose standard

Table 13: Rf value of extract and the standards from chromatography

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylulose</td>
<td>0.37</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.19</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.31</td>
</tr>
<tr>
<td>Sample(extract)</td>
<td>0.30</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.344</td>
</tr>
</tbody>
</table>

Legend: The Rf value of the sample corresponds to that of glucose standard
Antioxidant Activity of EEMC using FRAP, DPPH and Reducing power assay

Figure 30: Antioxidant activity of 50%, 70% and 100% EEMC by FRAP method. Legend: 50% ethanolic extract had the highest antioxidant activity compared to 70% and 100% ethanolic extracts respectively by FRAP ($p = 0.004$). The inset shows the standard graph with $R^2 = 0.9926$

Figure 31: Antioxidant activity of 50%, 70% and 100% EEMC by DPPH method. Legend: 50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by DPPH method ($p = 0.079$). The inset shows the standard graph with $R^2 = 0.9644$
Figure 32: Antioxidant activity of 50%, 70% and 100% EEMC by Reducing Power Assay.

Legend: 50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by Reducing power assay \((p = 0.010)\). The inset shows the standard graph with \(R^2 = 0.9947\).

The data obtained showed that, the 50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by FRAP \((p = 0.004)\), DPPH \((p = 0.079)\) and Reducing Power Assay \((p = 0.010)\), which may be attributed to the presence of higher percentage of phenolic acid and flavanoid content in 50% EEMC (Fig 30, 31, and 32).

Figure 33: IC(50) of EEMC by DPPH method

Legend: 50% ethanolic extract has lowest IC\((50)\) value \((11.43 \mu g/ml)\) when compared to 70% and 100% ethanolic extract

The data obtained from the DPPH method showed that, the 50% \((11.43 \mu g/ml)\) EEMC has lowest IC\((50)\) value than 70% \((16.42 \mu g/ml)\) EEMC and 100% \((175.4 \mu g/ml)\) EEMC (Fig.33).
Anti-inflammatory Activity of EEMC using HRBC Membrane Stabilization Assay

Figure 34: Anti-inflammatory activity of 50%, 70% and 100% EEMC.

Legend: 50% ethanolic extract had the highest anti-inflammatory activity followed by the 70% and 100% ethanolic extracts respectively ($p=0.001$) using diclofenac sodium as positive control.

The data obtained showed that, the 50% ethanolic extract had the highest anti-inflammatory activity followed by the 70% and 100% ethanolic extracts respectively (Fig.34) ($p=<0.001$) using diclofenac sodium as positive control.

b) Cell Line Studies

SRB assay showed similar pattern of cell death in both during differentiation and after differentiation group. The data obtained showed that, a dose of around 1.560µg/ml is effective for the treatment of 3T3-L1 cell lines with minimal cell death. (Fig 35)
Figure 35: Cytotoxic effect of different concentration of 50% EEMC on 3T3-L1 pre-adipocyte cell lines

Legend: Dose depended response was seen with lower concentration showing no cell death and higher concentration showing maximum cell death

In adipogenesis assay dose depended response was seen on treatment of 3T3-L1 cells with different concentrations of 50% EEMC with lower concentration having more lipid accumulation when compared to higher concentration. (Fig 38)

Figure 36: Effect of different concentration of 50% EEMC on adipogenesis in 3T3-L1 pre-adipocytes assessed by oil red staining

There was a dose depended response in 3T3-L1 cells for lipid accumulation when treated with increasing concentration of EEMC
Plotting the graph of different concentrations of 50% EECM against the percentage of adipogenesis showed that, on increasing the concentration of EEMC, there was a decrease in lipid accumulation in adipocytes when compared to the control, both during (Fig 39a) \((p<0.012)\) and after (Fig 39b) \((p<0.026)\) differentiation. However, the decrease in the percentage of adipogenesis was more during the differentiation when compared to after differentiation.

Figure 37: Graphical representation of the effect of different concentrations of 50% EEMC on adipogenesis during (a) and after (b) differentiation of 3T3-L1 pre-adipocytes

There was a dose dependent response in 3T3-L1 cells for lipid accumulation when treated with increasing concentration of EEMC both during and after differentiation

The glycerol release was estimated in the conditioned media after treating the cells with different concentrations of 50% EEMC during differentiation from day 0 to day 7. With an increase in the concentration of EEMC, there was a decrease in the glycerol release. This may be because the pre-adipocytes were treated with increasing concentration of EEMC from day 0 – day 7 and there was decreased lipid accumulation from lower to higher concentration. Less lipid accumulation in the higher concentration may be the reason for decreased glycerol release into the medium (Fig 40a) \((p<0.018)\).
However, when the cells were treated with different concentrations of 50% EEMC after differentiation there was an increase in glycerol release from lower to higher concentration. Here the pre-adipocytes were treated with different concentration of EEMC only after the cells were fully differentiated. The amount of lipid accumulated in them on the day 9 was almost similar in all the cells. Treating these cells with increasing concentration of EEMC resulted in increase glycerol release with the highest concentration of EEMC. (Fig 40b) \( p=<0.0007 \)

Figure 38: Effect of different concentration of 50% EEMC on adipolysis, during (a) and after (b) differentiation of 3T3-L1 pre-adipocytes

Treating with increasing concentration of 50% EEMC showed decreased glycerol release during differentiation and increased glycerol release after differentiation of 3T3-L1 preadipocytes. The inset shows the glycerol standard.
Figure 39: Quantitative analysis of Expression of PPARγ 2, PPARγ 1 and SREBP 1 Levels using ImageJ software (A). Western blot image (B).

There was dose dependent decrease in the expression of PPARγ and SREBP1 in the preadipocytes treated with Bitter melon or *Momordica charantia* with higher dose showing lower expression. The decrease in expression was more when treated with BM, during differentiation when compared to treatment after differentiation of the preadipocytes.

Treatment of the 3T3-L1 preadipocytes with BM resulted in an attenuation of the expression of adipogenesis-related factors and lipid metabolic genes. The expression of SREBP 1 and PPARγ, the central transcriptional regulators of adipogenesis, was decreased by the treatment with BM.
Figure 40: Real time PCR analysis of Expression of PPARγ 2, PPARγ 1 and SREBP 1
c) Animal Studies
In the present study the effect of fresh bitter melon juice on blood glucose, total cholesterol, triglycerides, adipocyte PPAR-γ and SREBP1 have been evaluated and its efficacy has been compared with that of Pioglitazone in streptozotocin induced diabetic rats.

The control group of rats showed minimal change in blood glucose levels. The diabetic control rats showed consistent hyperglycemia and the test drug showed a persistent decrease in the blood glucose level (BGL) from 1st to 28th day, while the standard drug did not show an appreciable decrease in BGL from 1st to 7th day but thereafter produced a persistent decrease in BGL up to 28th day.

In control group mean values of blood glucose levels range between 81 on day 0 to 79.5 mg/dl on day 28 without much of variation during the study. In untreated diabetic control rats, the blood glucose levels increased gradually from 341.2mg/ dl on 0 days to 430.5mg/dl on the 28th day. In Pioglitazone treated rats the mean blood glucose level was 370.5 mg/dl on 0 days, which showed a decrease only after the 7th day, then steadily decreased to 115.5 mg/dl on the 28th day, here there is a persistent reduction of blood glucose level from D1 to D 28. In *Momordica charantia* treated group the blood glucose level on day 0 was 363.3 mg/dl, which reduced to 318.5 on day 7 and later there was a persistent reduction of blood glucose level from 265.7 on day 14 to 147mg/dl on day 28 which was statistically significant compared to diabetic control (Table 14).

Table 14: MEAN±SD values of Blood Glucose levels in mg/dl in different groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>D0</th>
<th>D7</th>
<th>D14</th>
<th>D21</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81±5.76</td>
<td>81±4.733</td>
<td>79.83±6.14</td>
<td>80.83±5.529</td>
<td>79.5±4.183</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>341.2±24.64</td>
<td>368±36.57</td>
<td>394.5±44.94</td>
<td>410.3±49.43</td>
<td>430.5±58.45</td>
</tr>
<tr>
<td>Standard (Pioglitazone)</td>
<td>370.5±11.76</td>
<td>374±22.77</td>
<td>304.7±14.4</td>
<td>215.3±11.71</td>
<td>115.5±12.8</td>
</tr>
<tr>
<td>Test (BMJ)</td>
<td>363.3±10.67</td>
<td>318.5±15.03</td>
<td>265.7±14.75</td>
<td>220.2±14.08</td>
<td>147±17.33</td>
</tr>
</tbody>
</table>

The above data shows that there is good hypoglycemic effect of the herbal drug- *Momordica Charantia* (6 ml/kg body weight) and is comparable to that of the standard drug Pioglitazone (45 mg/kg body weight).

In the control group, no significant change in body weight throughout the study however in the diabetic control group, there was reduction of 20% body weight from day 1 to day 28 while in the standard group there was increase in body weight of up to 10% and in the test group there was slight reduction in body weight (Table 15).
Table 15: Mean body weight of rats in grams in different groups on different days

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>D0</th>
<th>D7</th>
<th>D14</th>
<th>D21</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>185±02</td>
<td>185±03</td>
<td>182±03</td>
<td>187±02</td>
<td>189±02</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>200±02</td>
<td>183±04</td>
<td>176±02</td>
<td>170±02</td>
<td>164±03</td>
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<td>Standard</td>
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<td>182±03</td>
<td>191±02</td>
<td>195±02</td>
<td>200±02</td>
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<tr>
<td>Test</td>
<td>188±02</td>
<td>184±03</td>
<td>190±04</td>
<td>189±02</td>
<td>180±02</td>
</tr>
</tbody>
</table>

Total cholesterol in the test group was 79.6 mg/dl and in the standard group it was 87 mg/dl it is decreased in both the groups when compared to diabetic control 109.8 mg/dl and normal control group 93.8 mg/dl. Triglycerides in test group was 86.3mg/dl and in standard group it was 85 mg/dl it is decreased in both the groups when compared to diabetic control 115.5 mg/dl and normal control group 98.5 mg/dl (Fig 35). PPARγ1, PPARγ2 and SREBP1 expression were decreased in test group when compared to diabetic control group (Fig 36).

![Sample Groups](image)

Figure 41: Bar graph showing comparison of Total Cholesterol and Triglycerides between different groups on day 28

The above data shows that there is good hypolipidemic effect of the herbal drug- *Momordica Charantia* (6 ml/kg body weight).
Genes of Interest (GOI)

Figure 42: Bar graph showing comparison of PPARγ1, PPARγ2 and SREBP mRNA expression between different groups on day 28