CHAPTER 7

PRECLINICAL PHARMACOKINETIC AND PHARMACODYNAMIC CORRELATION OF POLYHERBAL FORMULATION

7.1 INTRODUCTION

Pharmacokinetic (PK) and pharmacodynamic (PD) dosing parameter determine the relationship between drug concentration and efficacy that allow to determine with best treatment outcomes [110]. Improved herbal therapies for the treatment of various ailments are required to combat the global problem that lack proper scientific evidence for the herbal formulations to get the drug status in the global market. PK-PD correlation allows understanding of the meaning of blood toxicant concentration and thus improve its clinical use [111].

In the present study the PD of diabetic formulation was investigated by Streptozotocin (STZ) induced experimental diabetes. The cytotoxic action of STZ selectively destroys β-cells of pancreas by generating excess ROS and carbonium ion (CH₃⁺) leading to damage of DNA by alkylationing DNA bases causing oxidative damage [112]. PK studies were carried out for the optimal dose determined by PD activity in plasma and analyzed by validated HPLC-MS method.

The PK/PD correlations are explained by the maximum effect model (E_{max}) that correlates pharmacological response to drug concentrations was used to determine the maximum effect and the drug concentration that produces 50% maximum pharmacologic effect. E_{max} model describes the model mimics hyperbolic shape of the pharmacologic response – drug concentration curve and a maximum pharmacological response (E_{max}) may be induced by certain drug concentration beyond which no increase in pharmacological response will be obtained [113]. A PK/PD model with an effect compartment is used to describe the pharmacokinetics of the drug in the plasma and the time course of a pharmacologic effect of drug at site of action. The effect compartment is not part of the pharmacokinetics model but it is a hypothetical pharmacodynamic compartment that link to the plasma containing drug. Drug transfers from the plasma compartment to the effect compartment no significant amount of drug moves from the effect compartment to the
plasma compartment. Only free drug will diffuse into the effect compartment and the transfer rate constants are usually first order [114]

In the current research concerning correlation of dose-effect relationship and pharmacokinetic properties between single chemical compound and single therapeutic effect was developed to clarify mechanism of action of polyherbal formulation and to verify the role and contribution of Mangiferin in the US patented polyherbal formulation.

7.2 MATERIALS AND METHODS

The patented polyherbal formulation capsules batch number SRM001 was purchased from M/s Varanasi Bioresearch Pvt Ltd (Varanasi, India). Total cholesterol (TC), serum high-density lipoprotein (HDL), serum creatinine (SC), serum urea (SU), triglyceride (TG), serum glutamate oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) were assayed using standard kits from Erba Diagnostics (Mannheim, Germany). Streptozotocin and standard Mangiferin (99.04%) and gallic acid (99.98%) was purchased from Sigma Aldrich Co (MO, USA). Deionized water was produced by Milli Q system (Massachusetts). Acetonitrile (HPLC grade - Merck Co, India) and Formic acid (Rankem, India) were used for LCMS analysis.

7.3 EXPERIMENTAL PROTOCOL FOR PHARMACOKINETIC AND PHARMACODYNAMIC ACTIVITY

7.3.1 Pharmacodynamic activity on STZ induced experimental diabetes

Diabetes was induced by intra peritoneal (i.p) injection of streptozotocin at a dose of 120 mg/kg b.w. dissolved in 0.1 M cold citrate buffer (pH 4.5). STZ injected animals were given 20% glucose solution for 24h to prevent initial drug induced hypoglycemic mortality. Diabetes was confirmed one week after induction by measurement of tail vein blood glucose levels using glucose meter (Accu-check active, Roche, Diagnostics USA) by glucose oxidase-peroxidase method using strips. Animals with a blood glucose level above 200 mg/dL were considered diabetic and were used in experiment.
Animals were divided into six groups with six rats in each group.

Group I : Normal control (NC) rats received water (10ml/kg)

Group II : Diabetic control (DC) rats induced with STZ were treated with water (10ml/kg) for 28 days

Group III : Positive control (PC) rats induced with STZ were treated with Metformin (100 mg/kg) for 28 days

Group IV : Rats induced with STZ were treated with Polyherbal formulation (15 mg/kg equivalent weight of Mangiferin) (PF 15) for 28 days

Group V : Rats induced with STZ were treated with Polyherbal formulation (30 mg/kg equivalent weight of Mangiferin) (PF 30) for 28 days

Group VI : Rats induced with STZ were treated with Polyherbal formulation (60 mg/kg equivalent weight of Mangiferin) (PF 60) for 28 days

The freshly prepared solution of Metformin (0.5% CMC) and polyherbal formulation were orally administered once daily for 28 days. Body weights and blood glucose levels were measured on weekly on 12h fasted rats. On 14th day and at the end of the experimental period on 28th day, the animals were fasted overnight and blood was collected for biochemical estimation.

7.3.2 Estimation of Biochemical parameters

The serum lipid profile such as high density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG) were estimated using commercially available kits according to the manufacturer’s instructions (Erba Diagnostics) and measured on an auto analyzer (EM 360, Transasia, Mumbai).

7.3.3 Oral Glucose Tolerance Test (OGTT)

The rats were divided into four groups, with 6 animals in each group.

Group I: Normal rats treated with vehicle (NC)

Group II: Diabetic rats treated with vehicle (DC)

Group III: Diabetic rats treated with glibenclamide at dose of 10mg/kg (PC)

Group IV: Diabetic rats treated with polyherbal formulation 30mg/kg equivalent weight of Mangiferin. (PF 30)
After overnight fasting group III and IV rats were fed with glibenclamide and polyherbal formulation respectively. Normal untreated (group I) were fed with distilled water alone. Thereafter, following 30 min of post drug administration all animals were fed with glucose (2g/kg.b.wt). Blood samples were collected from tail veins prior to dosing and after 15, 30, 60, 120, 180, 240, 360 min of glucose administration.

7.3.4 Blood Glucose Monitoring

Blood glucose was monitored by tail vein blood collection. The tail tip was sanitized with alcohol and pricked and a drop of blood was used for blood glucose measurement using a glucometer (ACCU-Check, Roche Diagnostics Corporation, USA).

7.4 Histopathological Examination

For histopathological examination, kidney, liver, pancreas and spleen from each treatment group were fixed in formalin solution (10% v/v) for 24h, bisected longitudinally and embedded in paraffin. Sections of 4-6μm thickness were cut, stained by aqueous hematoxylin and alcoholic eosin and were examined by bright-field microscopy (Olympus, India)

7.5 Statistical Analysis

Statistical analyses were performed using Microsoft Excel (Microsoft, Seattle, Washington, USA). All data are presented as mean ± standard deviation (SD). Descriptive statistics of the concentration-time data of Mangiferin from formulation was carried out. Differences in the mean values were analyzed using ANOVA followed by Student’s t test. Significance is defined as p<0.05. In the analysis of the results of PK/PD correlation, the statistical comparison of area under curve (AUC) was performed after logarithmic processing.
7.6 RESULTS
7.6.1 Pharmacodynamic Activity
7.6.1.1 Effect of polyherbal formulation on bodyweight and blood glucose

The body weight of the diabetic control (Group 2) significantly decreased when compared to the normal control animals during the study (Group 1). Diabetic control continued to lose weight till the end of the study whereas the polyherbal formulation treated animals at all the three doses (15mg/kg, 30mg/kg and 60mg/kg equivalent weight of Mangiferin) showed significant improvement in body weight when compared to diabetic control (Table 7.1). Polyherbal formulation at a dose of 30mg/kg equivalent weight of Mangiferin shows significant increase in the body weight when compared with Metformin (100mg/kg) treated group.

Table 7.1. Effect of polyherbal formulation on body weight (g) in STZ – induced diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>0th Day</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
<th>28th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>208.3±12.18</td>
<td>212.1±12.79</td>
<td>225.7±12.06</td>
<td>238.6±12.04</td>
<td>250.4±13.28</td>
</tr>
<tr>
<td>DC</td>
<td>199.6±9.14</td>
<td>194.3±7.31</td>
<td>187.1±7.00</td>
<td>170.1±10.41</td>
<td>165.1±10.68†</td>
</tr>
<tr>
<td>PC</td>
<td>206.4±13.41</td>
<td>211.0±13.47</td>
<td>217.9±15.07</td>
<td>229.4±13.02</td>
<td>239.1±13.77**</td>
</tr>
<tr>
<td>PF-15</td>
<td>197.4±10.43</td>
<td>203.6±10.50</td>
<td>213.1±9.12</td>
<td>219.9±7.73</td>
<td>230.9±10.15**</td>
</tr>
<tr>
<td>PF-30</td>
<td>203.3±12.57</td>
<td>211±10.64</td>
<td>221.3±9.48</td>
<td>233.0±9.57</td>
<td>249.1±10.34**</td>
</tr>
<tr>
<td>PF-60</td>
<td>198.6±9.62</td>
<td>202.3±9.83</td>
<td>210.9±8.97</td>
<td>218.9±9.60</td>
<td>227.1±8.34**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD and n=6; Statistical analysis was by a one-way ANOVA with Turkey’s post-hoc test. † statistically different when compared with control group, p <0.001. * p < 0.001 with diabetic control, ** p < 0.01 with diabetic control, *** p < 0.05 with diabetic control.

Table 7.2. Effect of polyherbal formulation on blood glucose (mg/dL) in STZ – induced diabetes

<table>
<thead>
<tr>
<th></th>
<th>0th day</th>
<th>7th day</th>
<th>14th day</th>
<th>21th day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>81.70±5.36</td>
<td>85.15±5.28</td>
<td>84.88±2.30</td>
<td>87.08±5.49</td>
<td>89.88±5.61</td>
</tr>
<tr>
<td>DC</td>
<td>251.5±28.52</td>
<td>262.25±28.09</td>
<td>276.75±18.41</td>
<td>293.90±19.45</td>
<td>310.20±13.65†</td>
</tr>
<tr>
<td>PC</td>
<td>244.00±17.68</td>
<td>207.50±7.59</td>
<td>165.25±6.65</td>
<td>110.50±7.33</td>
<td>98.60±8.17†</td>
</tr>
<tr>
<td>PF-15</td>
<td>240.50±22.22</td>
<td>210.63±1.89</td>
<td>158.15±8.48***</td>
<td>135.90±10.30**</td>
<td>115.60±5.50††</td>
</tr>
<tr>
<td>PF-30</td>
<td>268.75±13.72</td>
<td>197.98±12.27</td>
<td>122.08±9.25**</td>
<td>106.95±12.75**</td>
<td>100.70±7.77††</td>
</tr>
<tr>
<td>PF-60</td>
<td>257.25±14.52</td>
<td>205.70±21.48</td>
<td>146.98±8.53***</td>
<td>120.75±12.90**</td>
<td>105.38±6.53**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD and n=6; Statistical analysis was by a one-way ANOVA with Turkey’s post-hoc test. † statistically different when compared with control group, p <0.001. * p < 0.001 with diabetic control, ** p < 0.01 with diabetic control, *** p < 0.05 with diabetic control.
The effect of the repeated oral administration of polyherbal formulation on blood glucose level in STZ induced diabetic rats was depicted in Table 7.2. In the present study, daily administration of polyherbal formulation at three different doses of 15mg/kg, 30mg/kg and 60mg/kg equivalent weight of Mangiferin to STZ-induced diabetic rats caused significant reduction on blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 28 (Table 7.2). Polyherbal formulation at a dose of 30mg/kg equivalent weight of Mangiferin exhibited maximum glucose lowering effect in diabetic rats when compared to the other two doses. Metformin (100 mg/kg) exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

7.6.1.2 Effect of polyherbal formulation on Lipid profile

The lipid profiles in control and experimental rats were depicted in Figure 7.1. In STZ induced diabetic rats, there was a significant increase in TC, TG, phospholipids, low density lipoproteins and significant decrease in HDL in serum compared to normal control. In diabetic rats, administration of polyherbal formulation of 30 mg/kg dose showed significant (P<0.05) reduction in elevated TC, TG, LDL and VLDL levels when compared to control rats. Also significantly (P<0.05) increased level of HDL was observed in diabetic rats treated with all doses of polyherbal formulation and Metformin compared to diabetic control rats.

![Fig 7.1](image.png)

Fig 7.1. Effect of polyherbal formulation on Lipid profile in STZ-induced diabetic rats. Each value is expressed as mean of six observations. † statistically different when compared with control group, p <0.05, **statistically different when compared with diabetic group p < 0.05
7.6.1.3 Effect of polyherbal formulation on Creatinine, SGOT, SGPT and urea levels

The efficacy of polyherbal formulation on serum SGOT, SGPT, Creatinine and urea in diabetic rat was represented in Table 7.3. The above biochemical parameters were altered in STZ induced diabetic rats compared to normal control rats. In diabetic rats, administration of polyherbal formulation and Metformin significantly reduced the SGOT, SGPT, creatinine and urea levels compared to diabetic control rats. The polyherbal formulation at a dose of 30 mg/kg equivalent weight of Mangiferin treatment showed significantly higher reduction of SGOT, SGPT levels compared to 15mg/kg and 60mg/kg equivalent doses of Mangiferin.

Table 7.3. Effect of polyherbal formulation on kidney and liver profile in STZ – induced diabetes

<table>
<thead>
<tr>
<th></th>
<th>SGOT(mg/dL)</th>
<th>SGPT(mg/dL)</th>
<th>Urea(mg/dL)</th>
<th>Creatinine(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14th Day</td>
<td>28th day</td>
<td>14th Day</td>
<td>28th day</td>
</tr>
<tr>
<td>NC</td>
<td>121.36±4.99</td>
<td>122.03±3.55</td>
<td>85.78±3.77</td>
<td>89.85±2.78</td>
</tr>
<tr>
<td>DC</td>
<td>209.91±13.94</td>
<td>220.37±12.78†</td>
<td>164.70±6.81†</td>
<td>176.34±5.43†</td>
</tr>
<tr>
<td>PC</td>
<td>122.09±8.20</td>
<td>121.04±9.59**</td>
<td>91.53±4.77</td>
<td>86.75±3.32**</td>
</tr>
<tr>
<td>PF 15</td>
<td>129.31±7.23</td>
<td>131.97±9.94***</td>
<td>103.81±9.82</td>
<td>100.12±4.22***</td>
</tr>
<tr>
<td>PF 30</td>
<td>122.98±9.38†</td>
<td>121.13±6.04†</td>
<td>92.70±5.39</td>
<td>92.27±3.73†</td>
</tr>
<tr>
<td>PF 60</td>
<td>126.90±11.35</td>
<td>124.03±11.05**</td>
<td>98.77±5.26**</td>
<td>99.19±4.41**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD and n=6; Statistical analysis was by a one-way ANOVA with Turkey’s post-hoc test. † statistically different when compared with control group, p <0.001. * p < 0.001 with diabetic control, ** p < 0.01 with diabetic control, *** p < 0.05 with diabetic control.
7.6.2 Histopathological examination

7.6.2.1 Histopathology of Liver

Histological appearance from the liver tissue section of control group showing normal hepatic structure (Figure 7.2A). Diabetic rats showed dilation in both central veins in the hepatic parenchyma and the portal veins and the bile ducts in the portal area. There were diffuse-mononuclear leucocytes inflammatory cells infiltration and Kupper cell proliferation in between the degenerated and fatty changed hepatocytes (Figure 7.2 B). These alterations were normalized in rats treated with polyherbal formulation and Metformin. Polyherbal formulation 30mg/kg equivalent weight of Mangiferin shows better recovery from the pathological changes induced by the STZ were depicted in Figure 7.2.

Figure 7.2. Histopathology of Liver: A: liver of control animal showing hepatic structure; B: liver of Diabetic animal showing severe fatty changes, sinusoidal dilation, feathery degeneration and necrosis; C: liver of diabetic animal treated with Metformin; D: liver of diabetic animal treated with polyherbal formulation of 15mg/kg equivalent dose of Mangiferin; E: liver of diabetic animal treated with polyherbal formulation of 30mg/kg equivalent dose of Mangiferin; F: liver of diabetic animal treated with polyherbal formulation of 60mg/kg equivalent weight of Mangiferin.
7.6.2.2 Histopathology of Kidney

Histological study of the normal kidney revealed normal glomerulus surrounded by the Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes (Figure 7.3A). The kidneys of untreated diabetic rats showed degenerated glomeruli infiltrated by the inflammatory cells and thickening of the basement membrane (Figure 7.3B). The proximal convoluted tubule exhibited edematous changes with deposition of mucopolysaccharide and hyaline substances. All the necrotic changes observed in the proximal and distal convoluted tubule along with the deposits were found to be absent in the diabetic rats treated with polyherbal formulation and Metformin (Figure 7.3C). The groups that were treated with polyherbal formulation showed features of healing i.e. normal glomerulus, absence of inflammatory cells, normal basement membrane and capillaries, decrease in the mucopolysaccharide and hyaline deposit, respectively.

Figure 7.3 Histopathology of the Kidney: A: Kidney of control animal; B: Kidney of Diabetic animal induced by Streptozotocin; C: Kidney of diabetic animal treated with Metformin; D: Kidney of diabetic animal treated with polyherbal formulation of 15mg/kg equivalent dose of Mangiferin; E: Kidney of diabetic animal treated with polyherbal formulation of 30mg/kg equivalent dose of Mangiferin; F: Kidney of diabetic animal treated with polyherbal formulation of 60mg/kg equivalent weight of Mangiferin.
7.6.2.3 Histopathology of pancreas

Histopathology of the pancreas (Figure 7.4) in control animals showed normal pancreatic parenchyma cells and islet cell. In diabetic control, pancreas section showed moderate hyperplasia of islet cells. Severe congestion in pancreatic parenchyma and mild infiltration of inflammatory cells. In diabetic animals treated with polyherbal formulation, Pancreas showed mild hyperplasia of islet cells and congestion of pancreatic parenchyma.

Figure 7.4. Histopathology of the Pancreas: A: Pancreas of control animal; B: Pancreas of Diabetic animal induced by Streptozotocin; C: Pancreas of diabetic animal treated with Metformin; D: Pancreas of diabetic animal treated with polyherbal formulation of 15mg/kg equivalent dose of Mangiferin; E: Pancreas of diabetic animal treated with polyherbal formulation of 30mg/kg equivalent dose of Mangiferin; F: Pancreas of diabetic animal treated with polyherbal formulation of 60mg/kg equivalent weight of Mangiferin.

7.6.2.4 Histopathology of spleen

The STZ-induced diabetic rats displayed partial injury and mild morphological changes in spleen (Figure 7.5). Diabetic rats treated with metformin showed normal architecture of all organs. Morphological changes in liver, kidney, pancreas and spleen due to direct toxic effect of streptozotocin as well as diabetic rats were remarkably reduces in rats treated with polyherbal formulation although unable to prevent them completely (15 mg/kg eq wt of Mgn of Polyherbal formulation) Islet hyperplasia (possibly due to β-cell hyperplasia) due to streptozotocin induced diabetes was less conspicuous in the rats treated with polyherbal formulation (15mg/kg eq wt of Mangiferin).
7.6.3 Effect of polyherbal formulation in the oral glucose tolerance test

Based on the sub chronic antidiabetic study for 28 days the dose of the polyherbal formulation was selected for the oral glucose tolerance test to elucidate the possible mechanism of the action of polyherbal formulation on the blood glucose. In the above study the oral dose of polyherbal formulation at a dose of 30 mg/kg equivalent weight of Mangiferin shows significant antidiabetic activity by lowering the blood glucose level and changes in the lipid profiles.

The data of the OGTT (Figure 7.6) revealed that the blood-glucose levels for experimental rats were increased at 60 min after glucose administration. Polyherbal formulation (30 mg/kg equivalent weight of Mangiferin) significantly inhibited the increase in blood glucose levels at 60, 120 and 180 min in glucose loaded rats when compared to the vehicle treated diabetic control rats than glibenclamide.
7.6.4 PK/PD Correlation

As shown in Figure 7.7, plasma Mangiferin concentration and polyherbal formulation effect showed a parallel time course profile. It suggested that Mangiferin was one of the major components in this polyherbal formulation and played an important role for antidiabetic activity of this formulation. Plasma Mangiferin concentration and blood glucose effect of this formulation showed a symmetrical manner in both high and low levels.

Table 7.4. Time courses of plasma Mangiferin concentration (µg/mL) and polyherbal formulation effect(%)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$C_p$ (µg/mL)</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.39±0.64</td>
<td>0.69±0.11</td>
</tr>
<tr>
<td>0.5</td>
<td>8.81±2.51</td>
<td>4.48±1.62</td>
</tr>
<tr>
<td>1.0</td>
<td>23.82±6.31</td>
<td>6.44±1.33</td>
</tr>
<tr>
<td>2.0</td>
<td>41.52±5.37</td>
<td>10.21±2.61</td>
</tr>
<tr>
<td>3.0</td>
<td>44.16±8.62</td>
<td>8.00±1.21</td>
</tr>
</tbody>
</table>
7.6.5 Maximum Effect ($E_{\text{max}}$) model

The maximum effect model is an empirical model that relates pharmacological response to drug concentrations. The $E_{\text{max}}$ model describes drug action in terms of maximum effect ($E_{\text{max}}$) and $EC_{50}$, the drug concentration that produces 50% maximum pharmacological effect.

$$E = \frac{E_{\text{max}}C}{EC_{50} + C}$$  \hspace{1cm} \text{Eq 7.1}

Where $C$ is the plasma Mangiferin concentration and $E$ is the pharmacological effect which was the response of polyherbal formulation on the blood glucose level. $EC_{50}$ was the drug concentration which produced 50% of $E_{\text{max}}$.

7.6.6 Sigmoid $E_{\text{max}}$ model

The above simple $E_{\text{max}}$ model will be used more for the drug showing the hyperbolic response-drug concentration curve. But the blood glucose effect – Mangiferin plasma concentration curve appears to the sigmoid curve rather than hyperbolic which can be best described by the sigmoid $E_{\text{max}}$ model is an extension model of the $E_{\text{max}}$ model. The equation for the sigmoid $E_{\text{max}}$ model is given in 7.2

$$E = \frac{E_{\text{max}}C^n}{EC_{50} + C^n}$$  \hspace{1cm} \text{Eq 7.2}

Where $n$ is an exponent describing the number of drug molecules that combine with each receptor molecule. Where $n$ is equal to unity ($n=1$), the sigmoidal $E_{\text{max}}$ reduces to the $E_{\text{max}}$ model. A value of $n>1$ influences the slope of curve and the model fit.

As shown in Figure 7.8 the concentration – response curve showed a counter clockwise hysteresis manner. A relationship was established between log of pharmacologic response and drug concentration in the effect compartment drug level. This hysteresis manner may be result of the Mangiferin being highly bound to the plasma protein which was explained by many studies [115] and of a slow initial diffusion into the effect compartment. A mathematical expression is used to describe the drug concentration to the time course and intensity of the pharmacologic response.

Considering the pharmacodynamic effect in the effect compartment produced by the Mangiferin, at steady state equilibrium, the clearance rate from the blood compartment
(A) to effect compartment (B) was equal to that form the effect compartment to blood compartment that was

\[
k_{1e} V_e = k_{eq} V_e \quad \text{Eq 7.3}
\]

Where \( V_e \) is volume of the effect compartment and \( k_{eq} \) is the elimination rate constant of the drug from the effect compartment. \( K_{1e} \) is the first order rate constant of the central compartment. And \( V_d \) is the volume of the central compartment.

The Mangiferin concentration in the effect compartment is calculated by using following formula

\[
C_e = \frac{A K_{eq}}{k_{eq} - a} \left( e^{-a t} - e^{-k_{eq} t} \right) + \frac{B K_{eq}}{k_{eq} - b} \left( e^{-b t} - e^{-k_{eq} t} \right) \quad \text{Eq 7.4}
\]

Where A and B are the two compartment model coefficients for the Mangiferin.

Main parameters calculated with the PK-PD model are listed in Table 7.5.

**Figure 7.7** Time course of Mangiferin concentration and polyherbal formulation effect in rats. *Filled diamond:* Concentration – time curve of Mangiferin (n=6). *Filled square:* Effects of polyherbal formulation on blood glucose (n=6)
Figure 7.8. Plasma Mangiferin concentration – Polyherbal formulation effect curve.

Table 7.5. Main parameters of Mangiferin with the PK-PD model in rats

<table>
<thead>
<tr>
<th>$K_e$ (1/h)</th>
<th>$K_a$ (1/h)</th>
<th>$V_d$ (L/Kg)</th>
<th>$EC_{50}$ (µg/mL)</th>
<th>$E_{max}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50 ± 0.15</td>
<td>0.36 ± 0.16</td>
<td>3.61 ± 1.04</td>
<td>29.12 ± 3.64</td>
<td>40.13 ± 5.37</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters including $K_e$, $K_a$ and $V_d$ were calculated according to data of plasma Mangiferin concentration after polyherbal formulation administration, with one compartment oral model; Pharmacodynamic parameters including $EC_{50}$ and $E_{max}$ were calculated according to effects of blood glucose levels taken after dosing of polyherbal formulation in rats with Scatchard method and Eq

**7.7 DISCUSSION**

Presently rational multidrug therapy is gradually increasing rather than single herbal regarding safety and efficacy aspects. But the question arises how does the polyherbal formulation shows its rational therapy. To elucidate the mechanism of actions of a combination of herbals and verify its rationality, the key is to investigate the active substance present in the preparation and its relationship between pharmacodynamics and pharmacokinetics. However due to the presence of multiple components the studies on mechanism and clinical medications are very difficult.

The present study was attempted to elucidate the possible mechanisms of the polyherbal formulation, a dose – effect relationship between the pharmacokinetic properties of active components and the pharmacodynamic effects induced by polyherbal formulation. It has been reported that Mangiferin has poor bioavailability in rats and
concentration is in range of µg/mL, when it was administered alone at a dose of 25 mg/kg [94]. We also conducted a study on this polyherbal formulation and found that the concentrations of Mangiferin, Ellagic acid and Hydroxycitric acid were 1.51, 0.91 and 5.33%w/w respectively [116]. On the basis of the method established in the assay a HPLC-MS method was optimized to quantify plasma Mangiferin concentration and its pharmacokinetics in rat was measured.

On the other hand the pharmacodynamic antidiabetic activity of this polyherbal formulation was studied at a dose of equivalent weights of Mangiferin in the formulation. Diabetes associated with weight loss. The reversal of weight loss in formulation treated diabetic group indicated that the restorative effect of this formulation may be by the reversal of gluconeogenesis and glycogenolysis [117, 118]. There was a significant rise in the concentration of lipids, such as cholesterol, triglycerides (TG) and HDL-C in diabetic rats than in the normal control group. Due to insulin deficiency the metabolic and regulatory mechanisms are altered which are responsible for the observed accumulation of lipids. Hypertriglyceridemia and hypercholesterolemia are major risk factors of diabetes mellitus involved in the development to atherosclerosis and coronary heart disease which are secondary complications of diabetes [119]. The results indicate that, polyherbal formulation administered during 28 days reduced total cholesterol, LDL-cholesterol, triglycerides and elevated HDL levels significantly at a dose of 30mg/kg equivalent weight of Mangiferin, when compared to other doses and Metformin. Thus as the patent information this formulation could have a potential to reduce long term cardiovascular complications associated with diabetes mellitus was validated.

Streptozotocin induces the elevation of biomarker enzymes such as SGOT, SGPT, urea creatinine was observed in diabetic control rats, and it indicate the hepatocellular damage. [120]. The hepatic damage was restored hepatocytes and the elevated transaminases were significantly reduced by polyherbal formulation. It was evident that the formulation also acting as hepatoprotective.

In the current study, a dose of 30mg/kg equivalent weight of Mangiferin in the polyherbal formulation was selected for the oral glucose tolerance test and pharmacokinetic study. It was found that this dose was optimal for hyperglycemia and elicits significant biochemical changes. Also it was reported that, Mangiferin doses beyond 100mg/kg exhibits behavioral signs of depression and its shows pharmacological activity.
when administered at a dose of 10 mg/kg through intraperitoneal route [121]. Thus, an oral route of administration with the dose level of 30mg/kg equivalent weight of Mangiferin was selected for the study. The studies on the hyperglycemic effect of the Mangifera indica suggested that the active principle might be Mangiferin responsible for glucose lowering action on oral glucose tolerance test [122]. In the present study the polyherbal formulation shows a significant changes in the blood glucose level during the course of time rather than Metformin, when compared to diabetic control group. Nevertheless chronic administration of this polyherbal formulation significantly improved the biochemical changes, lipid profile and oral glucose tolerance indicating its potent antidiabetic activity. These evidences prompted us to speculate the possible mechanism of action of this formulation like Metformin showing extra pancreatic actions and the other possible pancreatic mechanism like glibenclamide, i.e stimulating insulin release from the pancreatic β cells might contribute in improving oral glucose tolerance in the glucose – loaded diabetic rats and management of diabetic condition by preventing the further changes in metabolic and regulatory mechanisms. Taken together it may be summarized as, this polyherbal formulation possess both direct acting on the pancreas and extra pancreatic actions would be more advantageous to the existing oral antidiabetic monotherapy. These findings are consistent with results reported by Muruganandam et al. [121] regarding Mangiferin.

The results in the study revealed that, there is a relationship between Mangiferin plasma concentrations from this formulation to responses of blood glucose containing polyherbal formulation. It was suggested that, as major active component of Salacia species in this polyherbal formulation i.e. Mangiferin plays an important role in polyherbal formulation acting to lower the blood glucose levels for antidiabetic activity and management of vascular complications related to diabetes mellitus. The results also confirm that, Salacia species are the main herbs in this formulation to produce primary action and that composition of this polyherbal formulation is reasonable. Correlation between the pharmacokinetic and pharmacodynamic activity was established to describe the time course of this effect of polyherbal formulation effect and to reveal quantitative relationship between this polyherbal formulation effect and plasma Mangiferin levels. A PK/PD model with an effect compartment was used to describe the pharmacokinetics of the drug in the plasma and the time course of a pharmacologic effect of a drug in the site of action. A counter clockwise hysteresis manner developed in this model is the result of the
drug being highly bound to the plasma protein (α1 – acid glycoprotein) and slow initial diffusion of drug into the effect compartment [114]. Many literatures reported that, Mangiferin having higher binding effect with human serum albumin results in low bioavailability. The above results provided evidence for its binding activity of Mangiferin with human serum albumin. Investigations on the relationship between the responses and the active components are of benefit to explore the mechanisms of action and combination for polyherbal formulations.

7.8 CONCLUSION

In conclusion, there is a correlation between plasma concentration of Mangiferin concentrations and effect of plasma containing Mangiferin of this polyherbal formulation, which means that Mangiferin plays an important role in antidiabetic effects of this polyherbal formulation and the investigation on dose-effect relationships has displayed a feasible method to clarify mechanisms of combination for this patented polyherbal formulation.