Effects of Gymnema sylvestre extract on the pharmacokinetics and pharmacodynamics of Glimepiride in streptozotocin induced diabetic rats

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PII: S0009-2797(15)30143-5
DOI: 10.1016/j.cbi.2015.12.008
Reference: CBI 7549

To appear in: Chemico-Biological Interactions

Received Date: 9 May 2015
Revised Date: 24 November 2015
Accepted Date: 17 December 2015


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Abstract

*Gymnema sylvestre*, important Indian traditional herbal medicine has been used for diabetes from several years and marketed as single or multi-herb formulations globally. People are consuming *Gymnema. sylvestre* along with conventional hypoglycemic drugs. Therefore, there is need of evidence based assessment of risk versus benefits when *Gymnema. sylvestre* co-administered with conventional oral hypoglycemic drugs. In present investigation, pharmacodynamics and pharmacokinetic interactions with oral hypoglycemic drug, glimepiride (GLM) was studied in streptozotocin (STZ) induced diabetic rats. A specific and rapid HPLC-ESI-MS/MS method was established for simultaneous quantification of GLM and gymnemagenin (GMG) in rat plasma. Pharmacokinetic and pharmacodynamic interaction studies were carried out in STZ induced diabetic rats after concomitant administration of 400 mg/kg of *Gymnema. sylvestre* extract and 0.8 mg/kg of GLM for 28 days. The developed HPLC-ESI-MS/MS method was rapid, specific, and precise. Con-comitant oral administration of *Gymnema. sylvestre* extract (400 mg/kg) and GLM (0.8 mg/kg) in diabetic rats for 28 days showed beneficial pharmacodynamic interactions whereas no major alterations in the pharmacokinetics parameters of GLM and GMG were observed. This interaction demonstrated in animal model implies that significant clinical outcome might occur during concomitant administration of *Gymnema. sylvestre* extract and GLM especially in diabetic patients and warrants further studies in the same set up.

**Keywords:** *Gymnema sylvestre*, Glimepiride, Diabetes, Pharmacokinetic-pharmacodynamic interactions, LC-ESI-MS/MS.
1. Introduction

There is a general belief among the people that herbal drugs (HDs) are safe and helpful in reducing side effects associated with the conventional drugs. Although herbal products are becoming popular as alternative medicines worldwide, herb-drug and herb-herb interactions are a current topic of debate\(^1\). However, both the effectiveness of using herbal medicine in combination with modern pharmaceuticals, and the possible adverse effects from herb–drug interactions remain to be verified\(^2\). These studies are recommended by international regulatory agencies such as European Agency for Medicines (EMA) and US Food and Drug Administration (FDA) during the HD development process\(^3,4\). Recently several approaches such as use of *in silico*, *in vitro* tissue systems and *in vivo* models have been suggested for the evaluation of herb-drug interactions (HDIs) during drug development. In pharmacokinetic (PK) interactions, co-administered herb affects the absorption, distribution, metabolism or excretion of the conventional drug(s). Whereas, in pharmacodynamic (PD) interactions, co-administered herb has effects on targets such as receptors, enzymes, transcriptional factors or cytokines etc. leading to synergistic or additive or antagonistic or no effects of the therapy. Evidence based studies on risk versus benefit assessment after co-administration of herbs with commonly prescribed conventional drugs are lacking. Therefore, there is urgent need for systematic studies to address the potential benefit or risk associated with their con-comitant administration. *Gymnema sylvestre* (commonly named as Gurmar, family: Asclepiadaceae) is one of the well known plant used in diabetic conditions and is officially mentioned in Indian Pharmacopoeia\(^5\). *G. sylvestre* is the one of the major ingredient of various single as well as multi-herb formulations used to treat diabetic conditions globally\(^6,7\). Several preclinical studies on polar/non polar extract of roots and leaves of *G. sylvestre* suggested anti-hyperglycemic\(^8\), anti-hyperlipidemic\(^9\), antimicrobial\(^10\), anti-oxidant\(^11\), anti-inflammatory\(^12\) and anti-cancer activities\(^13\). Mechanistic
studies suggested that *G. sylvestre* exert its hypoglycemic effects through increase in insulin secretion \[14\], regeneration of islets cells, peripheral utilization of glucose, and inhibition of glucose absorption from intestine \[15\]. Gymnemic acids are acidic glycosides isolated from the leaves of *G. sylvestre* and have been reported as anti-sweet compounds, which can be quantified indirectly in terms of gymnemagenin (GMG), the hydrolyzed product of gymnemic acids\[16\]. This GMG has been reported as biomarker for pharmacokinetic studies after oral administration of *G. sylvestre* extract \[17\]. Considering the availability of gymnemagenin it was difficult to screen for its potential. Our group was successful in isolating the maximum yield and screened the same for its antidiabetic potential, for which an Indian patent has been filed\[18\]. Clinical reports validate the use of *G. sylvestre* in Type-1 and Type-2 diabetic conditions \[19\]. It has been consumed knowingly or unknowingly by the patients along with conventional oral hypoglycemic agents and could leads to potential drug interactions \[20\]. We have recently published *in silico* based evaluation of HDIs between *G. sylvestre* and GLM elsewhere \[21\]. We report here an impact of chemically standardized alcoholic extract of *G. sylvestre* on pharmacokinetic and pharmacodynamics (PK-PD) of glimepiride (GLM) as model oral hypoglycemic agent in streptozotocin (STZ) induced diabetic rats after 28 days of concomitant administration.

2. Materials and Methods

2.1. Drugs, chemicals, solvents

*G. sylvestre* leaf extract was kindly provided as a gift sample by M/s Natural Remedies Pvt. Ltd, Bangalore, India. Standard gymnemagenin (GMG) was purchased from Natural Remedies Pvt. Ltd, Bangalore, India. Glimepiride was provided as gift sample from Ranbaxy research laboratories, India. Withaferin A was purchased from ChromaDex (Laguna Hills, CA, USA,) and used as internal standard. Diagnostic kits for fasting blood glucose level (FBGL), Total cholesterol (TC) and Triglycerides (TG) estimation were purchased from Biolab diagnostic Pvt.
2.2. Chemical standardization of *Gymnema sylvestre* extract

*G. sylvestre* extract was standardized on the basis of GMG using previously reported high performance liquid chromatography with tandem mass spectrometry (HPLC–ESI–MS/MS) method\(^{[22]}\).

2.3. Pharmacokinetic interactions of *G. sylvestre* with glimepiride in diabetic rats

2.3.1. Animals

*Wistar* male rats, weighing 250 ± 25 gm were maintained at the animal house of JSS College of Pharmacy, Ootacamund, India. The animals were housed in standard conditions of temperature (22 ± 5°C), humidity (55 ± 15 %) and in 12 h light-dark cycles. They were fed on conventional laboratory pellet diet and water *ad libitum*. All the procedures were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Animal Welfare Division, Government of India, New Delhi, and was approved by the Institutional Animal Ethical Committee of J.S.S College of Pharmacy (JSSCP/IAEC/PH.COG/PH.D/04/2012-13), Ootacamund.

2.3.2. Induction of Diabetes

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared STZ (40 mg/kg bw) in 0.1M citrate buffer (pH 4.5) in a volume of 1 mL/kg bw. Diabetes was established in these STZ treated rats over a period of 4 days. After 4 days, the plasma glucose level of each rat was determined by tail vein puncture method using one touch simple select glucometer.
(Johnson & Johnson, Mumbai, India). Rats with a fasting plasma glucose range between 200-300 mg/dL were considered as diabetic.

2.3.3. Animal study

Thirty rats were randomly divided in their respective treatment groups (Six rats in each group). Excluding diabetic and normal control groups, rats were gavaged with GLM alone at 0.8 mg/kg dose in one group, *G. sylvestre* extract 400 mg/kg alone in second group and concomitantly administered both the drugs in third group, consecutively for 28 days. To avoid any physico-chemical influence on GLM absorption, GLM was given to all the animals by gavage an hour later. Highest dose for GLM in human is 8 mg[23] whereas for *G. sylvestre* extract it was reported in the range of 0.4-2.0 g/day[24,25]. According to the dose translation from animal to human studies, we used the body surface area (BSA) normalization method to convert the dose from human to rat[26]. FBGL was monitored on day 0, 7, 14, 21 and 28. Other parameters such as HbA1c, TC, and TG were measured on 28th day. The time intervals were selected with commonly used strategies for pharmacokinetic studies knowing the total blood consumption should not be over 20 % of the body blood volume[27]. Animals were anaesthetized with light ether anesthesia and blood was collected from retro-orbital route (0.4-0.5 mL) in EDTA vacutainer (3 mL, BD Biosciences). Sampling intervals were 0, 0.5, 1, 2, 3, 5, 10, 24, 48 and 72 h after dose administration. Blood samples were centrifuged at 7000 g for 15 min at 4°C to obtain the plasma which were stored at -70 °C until analysis using HPLC-ESI-MS/MS method. Animals were bleed at suitable intervals for evaluation of pharmacodynamic interactions of *G. sylvestre* and GLM concurrently administered in STZ induced diabetic animals on 7, 14, 21 and on 28th day for FBGL and body weight and on 28th day for HbA1c, serum insulin, TG and TC.
2.3.4. Instrumentations and analytical conditions

Sample analysis was carried out using HPLC-MS system consists of liquid chromatography (Shimadzu Prominence, Japan), a binary gradient pump (LC-20 AD), an autosampler (SIL HTC) and column oven (CTO-10ASVP). Chromatographic separation was achieved on a Luna C-18 column (150 mm x 4.6 mm; 5 mm particle size; Phenomenex, Torrance, CA, USA). The detection system used was triple quadrupole mass spectrometer (API 4000; Applied Biosystems/MDS SCIEX, CA, USA) with multiple reactions monitoring (MRM). The quantification of the analyte was performed using MRM acquisition due to the high selectivity and sensitivity. Dual switch mode such as negative and positive was used for GMG and Withaferin A respectively, whereas ionization of glimepiride (GLM) was carried out in positive mode.

2.3.5. Measurement of GMG and GLM in plasma samples using HPLC-ESI-MS/MS method

We developed a new HPLC-ESI-MS/MS method for simultaneous quantitative measurements of GMG and GLM in rat plasma samples with minor modifications from our previously published report on GMG [28]. Withaferin A was used as internal standards (IS). Primary stock solutions for GLM, GMG and internal standards (1mg/mL) were prepared in methanol. Working stock solution were prepared from primary stock using diluents methanol and water (40:60 v/v) to produce concentration of 10-1000 ng/mL and 100-6120 ng/mL for GLM and GMG respectively. Organic solvents were maintained less than 2% in the final volume. Chromatographic conditions including column, mobile phase composition and use of modifier were selected on the basis of our previously published method on estimation GMG in rat plasma and previously reported HPLC-MS/MS methods for estimation of GLM in plasma samples [29,30]. These methods suggest the use of C18 and CN column for optimal separation. Therefore, C18 column was selected towards the separation of GMG, GLM and IS. Towards, mobile phase optimization, we have
tried different composition of solvents such as water, methanol, acetonitrile and modifiers such as formic acid and ammonia. Isocratic elution of methanol and water (60:40 % v/v) with 0.1% formic acid and 0.3% ammonia resulted better resolution and good sensitivity. Plasma samples such as drug free plasma, calibration, QC and pharmacokinetic study samples were extracted using liquid-liquid extraction method. Briefly 100 µL of the aliquot and 25 µL of internal standard (150 ng/mL of Withaferin-A) were mixed and vortexed for 30 seconds to which 1 mL of ethyl acetate was added and vortexed it for another 2 min at 2500 rpm. The solution was centrifuged for 5 min at 4500 rpm. The aliquot supernatant was separated and evaporated. The evaporated aliquot supernatant was reconstituted with 100 µL of mobile phase (methanol: water, 60:40 v/v). The solution was vortexed for 2 min and 20 µL was injected into the HPLC-MS/MS system for simultaneous estimation of GMG and GLM. The method was thoroughly validated as per USFDA guidelines on bio-analytical method validation for selectivity, linearity, accuracy and precision[31].

2.4. Pharmacodynamic interactions in streptozotocin induced diabetic rats

2.4.1. Estimation of FBGL, HbA1c, serum insulin, TC, TG levels and body weight of animals

Various biochemical parameters such FBGL, TC and TG levels in pharmacodynamic study samples were estimated using commercial kits as per manufacture’s protocol. HbA1c and serum insulin estimation was carried out using ion exchange resin method [32] and insulin radioimmunoassay kit[33] at Plus Pathology Laboratory, Pune, India.

2.4.2. Histopathology of rat pancreas

Rat pancreas were removed immediately from the animals after sacrificing and rinsed with ice-cold saline. The tissues were fixed with 10% formaldehyde, dehydrated in a graded series of ethanol and embedded in paraffin wax before sectioning. Pancreas histopathology was carried
out at Plus pathology laboratory, Pune, India. The sections were stained in hematoxylin and
eosin (H & E). The photomicrographs of the each tissue section were observed using imaging
software for laboratory microscopy (Olympus, Tokoyo, Japan). Pathological grading was done
the basis of the extent of necrosis of islet cells as follows: ‘0’- normal, ‘+’- 0-25 % necrosis,
‘++’- 25% - 50% necrosis and ‘+++’- 50% -75% necrosis.

2.5. Statistical analysis
PD study data was expressed as mean ± standard error mean (SEM). Statistical analysis was
carried out by One-way ANOVA with post hoc Tukey’s test performed using Graph Pad Instat
software (version 3, San Diego, California, USA). p<0.05 was considered as level of
significance. The PK parameters such as area under the plasma concentration-time curve (AUC),
terminal elimination half-life ($t_{1/2}$) and oral clearance (CL) were estimated using pharmacokinetic
program, WinNonlin version 3.0 (Pharmasight corporation, Mountain view, CA). The maximum
plasma concentration ($C_{max}$) and the time to reach $C_{max}$ ($T_{max}$) were obtained directly from the
plasma concentration-time curve. All values were expressed as mean ± standard deviation (S.D.)
except $T_{max}$ which was mentioned as median. One-way ANOVA followed by Dunnett’s multiple
comparison tests was used for statistical comparison, taking p < 0.05 as significant.

**Results**

3.1. PK interactions of *G. sylvestre* extract with GLM

3.1.1. HPLC-ESI-MS/MS method for GMG and GLM

HPLC-ESI-MS/MS method was developed and validated for simultaneous determination of
GMG and GLM in rat plasma samples. The retention time for IS, GMG, and for GLM were
found to be 4.39 min, 4.66 and 5.06 min respectively (**Figure 1**). The method was validated for
specificity, linearity, accuracy, precision and recovery. The calibration curves showed good
linear correlation \( (r^2 \geq 0.996) \) between the concentration ranges of 0.5-50.00 and 5.00-306.00 ng/mL for GLM and GMG, respectively.

![Figure 1.](image)

**Figure 1.** Representative HPLC-ESI-MRM chromatograms of GLM and Withaferin A (IS) and GMG spiked in blank plasma and detected in study samples. Blank plasma did not show the presence of any peak at retention times of GLM \( (t_R = 5.06 \text{ min}) \) and GMG \( (t_R = 4.66 \text{ min}) \) respectively.

Intra and inter-day precision of the method was within the acceptable limits for GMG and GLM, it was ranged between 2.13-6.67 % and 2.07-8.26 %; and 2.39-6.03 % and 3.87-5.73 % respectively with R.S.D. < 10 % (Table 1).

**Table 1.** Precision and accuracy for GLM

<table>
<thead>
<tr>
<th>QC (ng/ml)</th>
<th>Intra-day (n=5)</th>
<th>Inter-day (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.56</td>
<td>0.50</td>
</tr>
<tr>
<td>1.25</td>
<td>1.31</td>
<td>1.25</td>
</tr>
<tr>
<td>25.07</td>
<td>26.41</td>
<td>25.07</td>
</tr>
<tr>
<td>37.69</td>
<td>37.14</td>
<td>37.69</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.567</td>
<td>0.567</td>
<td>0.567</td>
</tr>
<tr>
<td>1.29</td>
<td>1.29</td>
<td>1.29</td>
</tr>
<tr>
<td>25.66</td>
<td>25.66</td>
<td>25.66</td>
</tr>
<tr>
<td>37.44</td>
<td>37.44</td>
<td>37.44</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.41</td>
<td>5.72</td>
<td>5.73</td>
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<tr>
<td>5.72</td>
<td>5.72</td>
<td>5.72</td>
</tr>
<tr>
<td>6.03</td>
<td>6.03</td>
<td>6.03</td>
</tr>
<tr>
<td>5.73</td>
<td>5.73</td>
<td>5.73</td>
</tr>
<tr>
<td>5.25</td>
<td>5.25</td>
<td>5.25</td>
</tr>
<tr>
<td>RE (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.93</td>
<td>13.19</td>
<td>12.93</td>
</tr>
<tr>
<td>4.68</td>
<td>2.91</td>
<td>4.68</td>
</tr>
<tr>
<td>5.37</td>
<td>2.38</td>
<td>5.37</td>
</tr>
<tr>
<td>-1.46</td>
<td>-0.66</td>
<td>-1.46</td>
</tr>
</tbody>
</table>

**Table 1.** The \% CV and \% RE were considered as measures of precision and accuracy of the method respectively. Four different QC samples were prepared and analyzed within the same day \( (n = 5) \) and at different days \( (n = 15) \) for the measurement of precision and accuracy. \% CV and the \% RE were found within the acceptable limits.
The accuracy of the method was found within ± 15%. The developed method was found to be sensitive, selective, linear, precise and accurate for simultaneous quantitative estimation of GLM and GMG in rat plasma samples. The method was used for quantitative analysis of GLM and GMG in rat plasma samples obtained from herb-drug interaction studies.

3.1.2. Effect of *G. sylvestre* extract on GLM pharmacokinetic in STZ induced diabetic rats

Effect of sub-chronic administration (28 days) of *G. sylvestre* extract at a dose of 400 mg/kg on pharmacokinetic of GLM was studied in STZ induced diabetic rats. Pharmacokinetic profiles of GLM and GLM administered along with *G. sylvestre* extract showed almost superimposable curves (Figure 2).

![Figure 2](image_url)

**Figure 2.** Mean plasma concentration-time profiles of GLM after oral administration of GLM alone at a dose of 0.8 mg/kg and concomitant administration with *G. sylvestre* extract at a dose of 400 mg/kg. Values are expressed as mean ± SD (n=6).

AUC\textsubscript{0-72h}, C\textsubscript{max}, and t\textsubscript{1/2}, were found to be 2022.86 ± 117.96 ng*hr/mL, 413.21 ± 31.80 ng/ml and 14.67±1.10 hr GLM group respectively (Table 2) and does not show any significant change when compared with pharmacokinetics parameters obtained after concomitant administration of extract in STZ induced diabetic animals.
Table 2. Effect of *G. sylvestre* extract on GLM pharmacokinetics in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GLM alone</th>
<th>GLM + extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0-72hrs&lt;/sub&gt; (ng*hrs/mL)</td>
<td>2022.86 ± 117.96</td>
<td>1814.57 ± 10.99&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>413.21 ± 31.80</td>
<td>388.44 ± 25.11&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>14.67 ± 1.10</td>
<td>12.65 ± 0.29&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD (n = 6). C<sub>max</sub> (ng/mL): peak plasma concentration. AUC<sub>0-t</sub> (ng*hrs/mL): area under the curves from time zero to last interval and t<sub>1/2</sub> (h): terminal elimination half life. Mean values of control group (GLM alone) were compared with extract treated group. P≤0.05 was considered as level of significance.

3.1.3. Effect of GLM on GMG pharmacokinetic in STZ induced diabetic rats

Effects of sub-chronic treatment of GLM on pharmacokinetics of GMG from *G. sylvestre* were also studied in the same study model. Major pharmacokinetics parameters such as AUC<sub>0-72hr</sub>, C<sub>max</sub>, and t<sub>1/2</sub>, were found to be 6382.61± 652.49 ng*hr/mL, 620.24 ± 49.85 ng/mL and 13.04 ± 0.21 h for GMG after administration of *G. sylvestre* extract respectively (Table 3).

Table 3. Effect of GLM on pharmacokinetics of GMG after administration of *G. sylvestre* extract in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Extract alone</th>
<th>GLM + extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0-72hrs&lt;/sub&gt; (ng*hrs/mL)</td>
<td>6382.61± 652.49</td>
<td>7447.10 ± 117.56&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>620.24 ± 49.85</td>
<td>720.23 ± 69.87&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>13.04 ± 0.21</td>
<td>15.43 ± 0.97&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

It does not show any significant change when compared with pharmacokinetic parameters (Figure 3) obtained after concomitant administration of *G. sylvestre* extract and GLM in STZ induced diabetic animals.
3.2. PD interactions of *G. sylvestre* extract and GLM in STZ induced diabetic rats

3.2.1. Effect of extract, GLM and their concomitant administration on mean percentage change of FBGL

Results indicated that sub-chronic oral administration of the *G. sylvestre* extract (at 400 mg/kg dose) for 28 days resulted decrease in FBGL by 48.00% when compared to initial glucose value (at 0 day). GLM alone at a dose of 0.8 mg/kg also reduced FBGL to 57.73% after 28 days of administration in comparison with its initial values. Interestingly, con-comitant administration of extract with GLM resulted significant decrease in FBGL from 7th day and showed maximum reduction of 63.38% after 28 days of administration (Figure 4).

![Figure 3](image3.png)

**Figure 3.** Mean plasma concentration-time profiles of GMG after oral administration of extract at a dose of 400 mg/kg and concomitant administration with GLM at a dose of 0.8 mg/kg. Values are expressed as mean ± SD (n=5).

![Figure 4](image4.png)

**Figure 4.** Effect of GLM, extract and their concomitant administration on mean percentage change in values of fasting blood glucose level (FBGL) when compared with their initial values (Day 0) in STZ induced diabetic rats. Values are expressed as mean ± SE.
3.2.2. Effect of *G. sylvestre* extract, GLM and their concomitant administration on glycosylated haemoglobin (HbA1c) and serum insulin level

STZ induced diabetic rats showed significant increase (p<0.001) in HbA1c level and decrease in serum insulin level when compared to normal animal levels (Figure 5). GLM at a dose of 0.8 mg/kg showed decrease in HbA1c level (5.32%) when compared with untreated diabetic control (9.01%) after 28 days of administration.

![Figure 5](image)

*Figure 5.* Effect of 28 days of oral administration of GLM, extract and their concomitant administration on glycosylated haemoglobin (HbA1c) and serum insulin levels. Values are expressed as mean ± SEM (n=6); *p* value <0.05 considered as significant; ***p***<0.05, ****p***<0.01, *****p***<0.001 compared to the normal control group; ***p***<0.05, ****p***<0.01 and *****p***<0.001 compared to the untreated diabetic control group.

*G. sylvestre* extract at a dose of 400 mg/kg resulted in HbA1c level of 6.89 % when compared with untreated diabetic levels. Concomitant administration of GLM and extract resulted in significant decrease of HbA1c level to 4.82 %. The serum insulin level in diabetic group was decreased significantly (p<0.001) compared to normal control group. In diabetic rats, treatment with the extract and GLM showed increase in serum insulin level to 2.09 ± 0.12 and 1.99 ± 0.09 IU/mL from an initial value of 1.25 ± 0.12 IU/mL respectively. Concomitant administration of extract and GLM exhibits significant increase (p<0.001) in insulin level to 2.35 ± 0.11 IU/mL when compared with diabetic group insulin level.
3.3.3. Effect of *G. sylvestre* extract, GLM and their concomitant administration on TG and TC levels

The plasma TC and TG levels were significantly increased (p<0.001) in diabetic rats as compared to normal control rats. *G. sylvestre* extract at a dose of 400 mg/kg decreased plasma TC and TG level to 101.20 ± 5.52 mg/dL and 87.40 ± 4.25 mg/dL as compared to diabetes control values of 179.5 ± 5.05 mg/dL and 111.25 ± 6.13 mg/dL respectively after 28 days of administration (*Figure 6*). GLM at a dose of 0.8 mg/kg reduced plasma TC and TG level to 97.75 ± 3.05 mg/dL and 85.98 ± 2.69 mg/dL respectively as compared to level of diabetic control.

![Figure 6](image-url)

*Figure 6.* Effect of *G. sylvestre* extract, GLM and their concomitant administration on plasma total cholesterol(TC) and triglycerides (TG) level. Values are expressed as mean ± SEM (n=6); *p* value <0.05 is considered as significant; *p*<0.05, ***p***<0.01, ****p***<0.001 compared to the normal control group; *p*<0.05, ***p***<0.01 and ****p***<0.001 compared to the diabetic control group.

Subsequently, concomitant administration of *G. sylvestre* extract and GLM reveals significant decrease (p<0.001) in both plasma TC and TG levels to 89.1 ± 6.13 mg/dL and 72.25 ± 3.89 mg/dL respectively when compared with diabetic group and was found comparable with normal group values (82.4 ± 2.50 mg/dL and 69.6 ± 3.56 mg/dL).
3.3.4. Effect of extract, GLM and their con-comitant administration on body weight of animals

*G. sylvestre* extract and GLM alone result showed an increase in the body weight in comparison to their basal weight at a dose of 400 mg/kg and 0.8 mg/kg respectively after 28 days of oral administration in diabetic rats. Concomitant administration of both *G. sylvestre* extract and GLM enhanced the body weight by -0.86 % and found significant (p<0.01) when compared to the diabetic animals and the animals nearly restored to their initial weight (Figure 7).

![Figure 7. Effect of extract, GLM and their concomitant administration on body weight of animals.](image)

**Figure 7.** Effect of extract, GLM and their concomitant administration on the % change in body weight of animals. The percentage change in the body weight was calculated with respect to initial body weight (Day 0). Values are expressed as % change in body weight (g) ± SEM (n=5); p<0.05 is considered as significant; *p*<0.05, **p**<0.01, ***p***<0.001 compared to the normal control group; #p<0.05, ##p<0.01 and ###p<0.001 compared to the diabetic control group.

3.3.5. Effect of *G. sylvestre* extract, GLM and their concomitant administration on histopathology of pancreas

Histopathological changes of pancreas are given in (Figure 8). Based on tissue sections, normal control rats did not show any notable changes (Grade 0) in pancreas histology throughout the 28 days study. In contrast, STZ administration elicited severe injury to pancreas, leading to decrease in the islet cell numbers and in the diameter of pancreatic islets (Grade +++).
Figure 8. Effect of *G. sylvestre* extract, GLM and the concomitant administration on histopathology of rat pancreas. Pathological changes were graded and scored based on extent of tissue necrosis. Grade "0" indicates normal; Grade "+" indicates necrosis 0-25%; Grade "++" indicates necrosis 25-50%; Grade "+++" indicates necrosis 50-75%. Microscopic photographs: a: Untreated diabetic rat pancreas; b: Normal rat pancreas; c: Pancreas of animals treated with GLM (0.8 mg/kg); d: Pancreas of animals treated with *G. sylvestre* extract at dose of 400 mg/kg; e Pancreas of animals administered with GLM+*G. sylvestre*.

The islets were shrunken in diabetic rats when compared with normal rats. Administration with GLM (0.8 mg/kg), *G. sylvestre* extract (400 mg/kg) showed moderate expansion of islets and significantly reduced the injuries to pancreas within 28 days of treatment (Grade+++ respectively). The damage of pancreatic tissue was recovered in *G. sylvestre* treated diabetic group and GLM-treated diabetic group. GLM has also been reported to have insulin secretory activity and this might be from partially survived β-cells (remnant cells) after administration of STZ. These observations further support the previously published reports on GLM. GLM is a hypoglycemic agent which causes intensification of the insulin secretion by β-cells of the pancreas by closing up the potassium channels and depolarizing the cell membrane, and in consequence it initiates metabolic process which leads to release of insulin. Apart from above mentioned activities, GLM was also reported for its extra pancreatic activity. The concomitant administration of *G. sylvestre* extract and GLM group showed significant reduction in necrosis.
and regeneration of islet cells compared with untreated rat pancreas after 28 days of treatment (Grade +) as shown in microphotographs.

4. Discussion

It has been estimated that people living with diabetes are using complimentary alternative medicines (CAM) with predominant form of herb ranges from 17% to 73% \[^{36}\] \(G.\ sylvestre\), which is an important Indian traditional herbal medicine has been in use for diabetes from several years. People are consuming \(G.\ sylvestre\) along with conventional hypoglycemic drugs. Therefore, there is need of evidence based assessment of risk versus benefits when \(G.\ sylvestre\) is co-administered with conventional oral hypoglycemic drugs. The aim of the present investigation was to evaluate the pharmacokinetic and pharmacodynamic interactions of \(G.\ sylvestre\) extract with glimepiride (GLM), oral hypoglycemic drug, in STZ induced diabetic rats. The pharmacokinetics data obtained in the present study revealed that the AUC\(0-72\)hr, \(C_{\text{max}}\) and \(t_{1/2}\) of \(G.\ sylvestre\) extract, in terms of GMG, and GLM were not significantly affected by each other. Thus, there is no significant pharmacokinetic interaction between \(G.\ sylvestre\) extract and GLM.

On the contrary, an increased level of plasma glucose in STZ induced diabetic rats was significantly lowered by the concomitant administration of both \(G.\ sylvestre\) extract and GLM in dose and time dependent manner as compared to the individual drugs. The reduced glucose level suggested that both the co-administered herb and drug might exert their action through insulin release by the stimulation of a regeneration process and revitalization of the remnant beta cells. This was clearly evidenced by the significant increased levels of serum insulin in diabetic rats co-administered with \(G.\ sylvestre\) extract and GLM. Reports by 22 different groups of workers, using every variety of immunoassay measured fasting insulin concentration in serum and in plasma. It was observed that insulin level was higher in plasma than in serum\[^{37}\]. Researchers of these studies have concluded that when insulin is measured in blood it should be measured in
serum. Further, the concomitant administration of both *G. sylvestre* extract and GLM also restored the initial weight of animals and decreased both plasma TC and TG levels near to normal values. Con-comitant administration of extract along with GLM resulted significant decrease (*p*<0.001) in TG and TC levels although the detailed mechanism behind it needs to be studied, but there are reports about gymnemic acids, a class of triterpene saponins, considered as pharmacologically active constituents from *G. sylvestre* have high cholesterol-binding affinity, leading to an increase in its fecal excretion. Studies have been also reported about 55% reduction in TG has been produced by GLM. These reports support the observation that we have reported here after con-comitant administration of GLM and Gymnema extract. The level of HbA1c is monitored as a reliable index of glycemic control in diabetes as in uncontrolled diabetes there is an increased glycosylation of proteins like hemoglobin (Hb). Significant reduction in HbA1c levels indicates the efficiency of therapy in diabetic control. In the present study, the concomitant administration of both *G. sylvestre* extract and GLM significantly reduced the level of HbA1c level as compared to the individual drugs indicative of very prominent pharmacodynamic interactions taking place in concomitant administration of *G. sylvestre* extract and GLM. Conventional laboratory animals, rats, have been used to gain a better perceptive of the effect of sub-chronic con-comitant administration of *G. sylvestre* extract and GLM for 28 days resulted significant increase in anti-hyperglycaemic and anti-hyperlipidemic activities leading to pharmacodynamic interactions. However, this observation warranted further studies after chronic co-administration as controlled trials or case studies in human volunteers. Such case studies will be helpful in getting more convincing clinical data which can give more insight to patients administering such combination.
5. Conclusion

There are increasing incidences of herb-drug interactions leading to beneficial or unwanted effects. Therefore, in this work, effect of *G. sylvestre* extracts on pharmacokinetics and pharmacodynamic of conventionally used oral hypoglycemic, Glimepiride (GLM) was studied in STZ induced rats. Sub-chronic co-administration of extract and GLM for 28 days resulted significant increase in anti-hyperglycemic and anti-hyperlipidemic activities leading to pharmacodynamic interactions. The study suggested that extract or the GLM could be administered at lowered dose for better therapeutic potential and decreased side effects. However, a chronic administration may possibly cause hypoglycemia and therefore, this observation warranted further studies after chronic co-administration as controlled clinical trials or case studies.
References


18. Kamble B., Gupta A., Patil D., Pathak D., Duraiswamy B Pure gymnemagenin, or its salts, ethers or esters or its polymeric nanoparticles with potent antidiabetic and anti-hyperlipidaemic activity. Indian Patent Application No.4130/CHE/2012 A


Caption for Figures

**Figure 1.** Representative HPLC-ESI- MRM chromatograms of GLM, Withaferin A (IS) and GMG spiked in blank plasma and detected in study samples. Blank plasma did not show the presence of any peak at retention times of GLM ($t_R = 5.06$ min) and GMG ($t_R = 4.66$ min) respectively.

**Figure 2.** Mean plasma concentration-time profiles of GLM after oral administration of GLM alone at a dose of 0.8 mg/kg and con-comitant administration with *G. sylvestre* extract at a dose of 400 mg/kg. Values are expressed as mean ± SD (n=6).

**Figure 3.** Mean plasma concentration-time profiles of GMG after oral administration of extract at a dose of 400 mg/kg and con-comitant administration with GLM at a dose of 0.8 mg/kg. Values are expressed as mean ± SD (n=5).

**Figure 4.** Effect of GLM, extract and their concomitant administration on mean percentage change in values of fasting blood glucose level (FBGL) when compared with their initial values (Day 0) in STZ induced diabetic rats. Values are expressed as mean ± SE

**Figure 5.** Effect of 28 days of oral administration of GLM, extract and their concomitant administration on glycosylated haemoglobin (HbA1c) and serum insulin levels. Values are expressed as mean ± SEM (n=5); *p value <0.05 considered as significant; *p<0.05, **p<0.01, ***p<0.001 compared to the normal control group; #p <0.05, ##p<0.01 and ### p<0.001 compared to the untreated diabetic control group.

**Figure 6.** Effect of *G. sylvestre* extract, GLM and their concomitant administration on plasma total cholesterol (TC) and triglycerides (TG) level. Values are expressed as mean ± SEM (n=5); *p value <0.05 is considered as significant; *p<0.05, **p<0.01, ***p<0.001 compared to the normal control group; #p<0.05, ##p<0.01 and ### p<0.001 compared to the diabetic control group.

**Figure 7.** Effect of extract, GLM and their con-comitant administration on the % change in body weight of animals. The percentage change in the body weight was calculated with respect to initial body weight (Day 0). Values are expressed as % change in body weight (g) ± SEM (n=5); p<0.05 is considered as significant; *p<0.05, **p<0.01, ***p<0.001 compared to the normal control group; #p<0.05, ##p<0.01 and ### p<0.001 compared to the diabetic control group.

**Figure 8.** Effect of *G. sylvestre* extract, GLM and their concomitant administration on histopathology of rat pancreas. Pathological changes were graded and scored based on extent of tissue necrosis. Grade “0” indicates normal; Grade “+” indicates necrosis 0-25 %; Grade “++” indicates necrosis 25-50 %; Grade “+++” indicates necrosis 50-75 %. Microscopic photographs a: Untreated diabetic rat pancreas; b: Normal rat pancreas; C: Pancreas of animals treated with GLM (0.8 mg/kg); d: Pancreas of animals treated with *G. sylvestre* extract at dose of 400 mg/kg; e: Pancreas of animals administered with GLM+*G. sylvestre*. 
HIGHLIGHTS

- HPLC-ESI-MS/MS method, for simultaneous determination of GMG and GLM in rat plasma.
- No significant pharmacokinetic of *G. sylvestre* extract, in terms of GMG, and GLM.
- Significant pharmacodynamic interactions of *G. sylvestre* and GLM.
- This observation warranted further controlled clinical trials or case studies.