

# Effect of Ethanolic Extract of Aerial Parts of *Andrographis paniculata* on the Pharmacokinetics of Gliclazide in Rats.

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## Research Article

### Article Info:

Received on 11/11/2015

Accepted on: 04/12/2015

Published on: 23/12/2015



QR Code for mobile



### ABSTRACT :

Drug Interaction is the interaction between a drug and another substance that prevents the drug from performing as expected. Complementary and Alternative Medicines (CAM) mostly by herbs is often used concomitantly with conventional medicine and its use may not be disclosed by patient to their physician which may lead to Herb-Drug interactions. The present study aims to find out any possible interaction between the herbal drug i.e., 60% ethanolic extract of aerial parts of *Andrographis paniculata* on the anti-diabetic drug Gliclazide as these involve metabolic reactions using CYP2C9 and CYP3A4 enzymes. In this study, three groups of Albino wistar rats of either sex were used of which Group -I was used to determine the spiked serum concentration, group-II treated with oral Gliclazide (2mg/kg) and group-III with ethanolic extract of aerial parts of *Andrographis paniculata* (2gm/kg) orally for 7 days followed by Gliclazide (2mg/kg) orally on 8<sup>th</sup> day are used to determine the change in Pharmacokinetic parameters. Blood from different groups were collected from retro-orbital plexus at 1,2,3,4,6,8,12,18,24 hrs after Gliclazide administration and concentration of Gliclazide was determined by using HPLC. There was a significant increase in C<sub>max</sub>, K<sub>el</sub>, T<sub>1/2</sub>, AUC and the percentage increase in bioavailability of 63.39% was observed in extract pre-treated group when compared to Gliclazide alone treated group which suggests the decreased metabolism of Gliclazide by *Andrographis paniculata* involving CYP2C9 and CYP3A4 enzymes. Thus add-on preparations containing *Andrographis paniculata* may be cautiously used and dose adjustment strategies are to be adopted when administered with Gliclazide to minimize the adverse effects of Gliclazide.

**Key Words:** *Andrographis paniculata*, Gliclazide, CYP2C9, CYP3A4, Bioavailability.

## INTRODUCTION:

A drug interaction can be defined as an interaction between a drug and another substance that prevents the drug from performing as expected [1]. Drug interactions may be drug-drug or food-drug interaction. Drug development process should evaluate the new drug potential of metabolizing the other drugs i.e., to determine whether the drug is a substrate, inhibitor or inducer of metabolic enzymes.

Over a past decade, there has been increased global interest in Complementary and Alternative medicines (CAM) which is often used concomitantly with the conventional medicine. It is studied that many patients consume Herbal supplements along with Prescription drugs and many patients do not disclose this to their Physician which may lead to Herb drug interaction [2].

As herbal medicines are usually a mixture of many active ingredients, which can result in unexpected low/high concentrations of therapeutic drug and may lead to less effect/undesired side effects.

Herbal drug interactions are based on same pharmacokinetic and pharmacodynamics mechanisms as

Drug-drug interaction which may result in alteration in Plasma concentration of object drug and as a consequence may frequently lead to toxic or sub therapeutic levels depending on the metabolizing enzymes inhibited or induced. For instance, Lignans of *Piper cubeba* Linn. fruits inhibits CYP3A4 enzymes, thus inhibiting the metabolism of therapeutic drug and raising the plasma drug concentration [3].

Gliclazide is a second generation Sulphonyl urea which acts as a hypoglycemic agent. It stimulates  $\beta$  cells of the islet of Langerhans in the pancreas to release insulin. It also enhances peripheral insulin sensitivity. Gliclazide is metabolized by both CYP2C9 and CYP3A4 [4].

*Andrographis paniculata* belongs to family Acanthaceae and is called as "King of Bitters". It contains bitter constituents like andrographolide- a diterpene lactone, diterpenoids, deoxyandrographolide and other constituents like andrographolide D, homoandrographolide, andrographan, andrographon etc. The ethanolic extract of *Andrographis paniculata* inhibits the CYP2C9 and CYP3A4 enzymes in

doi: 10.15272/ajbps.v5i51.755

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Conflict of interest: Authors reported none

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hepatic microsomes of human [5].

Thus there are chances that this ethanolic extract of aerial parts of *Andrographis paniculata* may interact with Gliclazide as this is metabolized by the same CYP2C9 and CYP3A4 enzymes and inhibits the metabolism of Gliclazide to raise the Plasma Gliclazide concentration.

There is a dearth of literature for the interaction between ethanolic extract of aerial parts of *Andrographis paniculata* and Gliclazide. Hence the present study was carried out with main objective to evaluate any possible herb-drug interaction between ethanolic extract of aerial parts of *Andrographis paniculata*-the precipitant drug and Gliclazide –the object drug.

## MATERIALS AND METHODS

### Materials:

*Andrographis paniculata* plant was obtained from Forests near Tirupathi region, identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati.

Gliclazide obtained from MSN Laboratories Pvt.Ltd. The HPLC grade methanol and ammonium acetate (Merck, Mumbai, India). All other chemicals used were of analytical grade. The drug analysis was carried out using HPLC system (Shimadzu, Kyoto, Japan) having Rheodyne injector port (20 µl loop), and UV/VIS detector (SPD 10A Vp). The data interpretation was done with LC-solutions (Shimadzu, Kyoto, Japan) data acquisition software.

### Animals:

Albino rats of either sex, 3-4 months of age, weighing between 200 to 250 g, were used in the study. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 °C and 50 ± 15% relative humidity, with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet and water *ad libitum*. The food and water were withdrawn. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee for animal research. (Reg.No.51/01/C/CPCSEA/2011/09). The study was conducted in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Preparation of extract:

The extraction was carried out by mixing *Andrographis paniculata* powder with 1:3 w/v in 60% ethanol v/v using a Soxhlet apparatus for 12 h. The extract was filtered and the solvent from the filtrate was removed using a rotary evaporator under reduced pressure and low temperature. The extract was stored in an airtight container below 4°C [5].

### Phytochemical screening:

Phytochemical screening of the crude extract was carried out employing standard procedures.

### Sample collection:

Blood samples were collected from retro-orbital plexus.

### Acute Oral Toxicity Study:

Acute oral toxicity test was carried out according to the OECD guidelines 423. Female wistar albino rats (150-200 gm weight) were used. Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose (2000mg/kg body weight) of ethanolic extract of aerial parts of *Andrographis*

*paniculata*. After the administration of extract, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter for a period of 14 days.

### Selection of dose of the extract:

LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The biological evaluation can be carried out at doses of 200, 500, 1000, 2000 mg/kg body weight.

### Study design:

Pharmacokinetic study normal rats:

Rats were divided into 3 groups (n=6).

Group 1 - (Control) - Rats were orally administered with 0.9%w/v saline used to determine the Gliclazide Spiked serum concentration.

Group 2 - (Standard) - Rats were fasted overnight and were orally administered with Gliclazide at dose of 2mg/kg.

Group 3 - (Test) - Rats were fed orally with ethanolic extract of *Andrographis paniculata* at dose of 2 gm/kg for seven days followed by fasting overnight on seventh day and were orally administered with Gliclazide at dose of 2mg/kg on 8<sup>th</sup> day.

### Preparation of Mobile Phase:

The mobile phase is a mixture of phosphate buffer (PH-3.4) and acetonitrile in the ratio of 45:55 and it is filtered using vacuum for degassing before using.

### Preparation of stock solutions and working standard solutions:

Stock solutions of Gliclazide (100 µg/ mL in acetonitrile) were prepared and stored in refrigerator during the experiments. HPLC grade acetonitrile and double distilled water were used throughout the analysis. 100 µL of GL standard solutions at concentrations of 2, 4, 6, 8, 10, 12, 14 µg / mL and 20 µL injected into HPLC to know the specific drug peak at specific Retention time.

- HPLC: Shimadzu HPLC system was used with SUPELCOSIL LC-18 column quantified by UV detection at 269 nm at a flow rate of 1.2 ml/min.

### Method of extraction of Gliclazide from Plasma [6]:

#### Spiked Serum concentration:

50 µL of GL working solution, 100 µL of 0.07M phosphate buffer (pH=4.4) were added to 100 µL of serum. After vortex mixing for 10 sec, 1 mL of toluene was added and the mixture was shaken vigorously for 1 min and was centrifuged for 15 min at 10000 rpm (8500g). An 800 µL aliquot of the upper organic layer containing GL was transferred to a clean glass tube and evaporated under air stream to dryness at 50°C. The residue was re-dissolved in 100 µL of mobile phase and a 20 µL aliquot was injected onto the HPLC column to obtain Area at specific RT for different concentrations and linearity curve is plotted. The areas of peak to specific concentrations at specific RT in serum will serve as Serum Calibrators.

#### Extraction of Gliclazide from Serum from the extract and drug treated group:

#### Blood sample withdrawal:

About 0.3 ml of Blood is collected from Retro Orbital at 1,2,3,4,6,8,12,18,24 hrs in Eppendorf tubes and Serum

is separated by centrifugation at 10000 rpm (8500 g) for 15 minutes and stored at -20° c until analysis, and then extraction of Gliclazide is done as explained in determining Spike Serum concentration. Serum Gliclazide concentration was determined by comparing the Areas of peak obtained in chromatogram with Serum Calibrators (Spike Serum Concentration). The above collected blood samples were also used for determination of glucose by Glucometer or by GOD-POD method [7].

**Data analysis:**

Pharmacokinetic parameters are calculated by Method of Residuals and % increase in Bioavailability in Test treated group is determined. The maximum plasma concentration ( $C_{max}$ ), time needed to reach the maximum plasma concentration ( $T_{max}$ ), area under the concentration– time curve ( $AUC_{0-\infty}$ ), mean residence time (MRT), elimination rate constant ( $K_{el}$ ), clearance and half-life ( $T_{1/2}$ ) were calculated .

**Statistical analysis:**

All the means are presented with their standard deviation (mean  $\pm$ S.D). The pharmacokinetic parameters of Gliclazide groups and extract treated group were compared using paired Student’s t-test .P < 0.05 was considered statistically significant.

**RESULTS**

**Phytochemical screening:**

Phytochemical screening revealed the presence of diversified chemical constituents such as diterpenoid lactones, flavones and flavonoids. It has been reported that Andrographolide is the main diterpenoid present in the extract [8].

**Acute toxicity study:**

In these studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed.

**Selection of dose of the extract:**

LD50 was determined as per OECD guidelines for fixing the dose for biological evaluation and the maximum dose of 2 gm/kg body weight was selected.

**Pharmacokinetic interaction in normal rats:**

Mean pharmacokinetic parameters of Gliclazide in Test in normal rats were shown in Table 1. The pharmacokinetic parameters of Gliclazide like  $AUC_{0-\infty}$ ,  $C_{max}$ ,  $T_{1/2}$ ,  $K_{el}$  and MRT were altered significantly in rats treated with extract when compared to Gliclazide alone treated group shown in Table 1.

Chromatogram of Gliclazide in Serum when spiked at concentration of 10 $\mu$ gm/ml is shown in the Figure 1.

Mean serum concentration of Gliclazide in Gliclazide alone and extract pre-treated groups are shown in Figure 2.

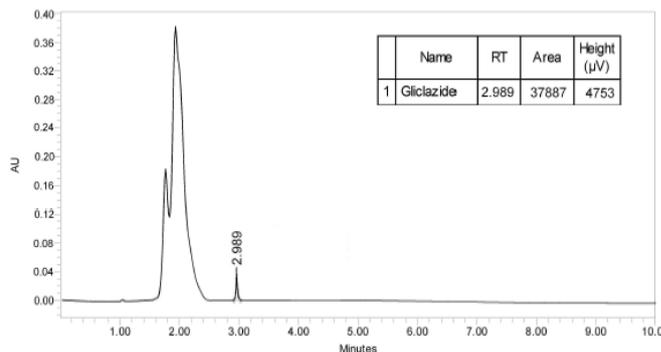


Figure 1. Chromatogram of Gliclazide in Serum when spiked at a concentration of 10 $\mu$ gm/ml.

Table 1. Mean pharmacokinetic parameters of Gliclazide alone and in presence of Extract in Normal rats

Pharmacokinetic Parameters	Gliclazide alone treated group	Extract+Gliclazide
$C_{max}$ ( $\mu$ g/ml)	3.42 $\pm$ 1.20	4.78 $\pm$ 1.69**
$T_{max}$ (h)	2.81 $\pm$ 0.05	2.84 $\pm$ 0.24 <sup>ns</sup>
$K_{el}$ (1/h)	0.078 $\pm$ 0.005	0.056 $\pm$ 0.0001***
Vd (ltr)	0.113 $\pm$ 0.06	0.08 $\pm$ 0.01 <sup>ns</sup>
Clearance (ml/h)	0.021 $\pm$ 0.028	0.0053 $\pm$ 0.0024 <sup>ns</sup>
T (h)	8.88 $\pm$ 0.56	12.18 $\pm$ 0.015***
$AUC_{0-\infty}$ ( $\mu$ g-h/ml)	46.10 $\pm$ 16.03	76.73 $\pm$ 27.17**
AUMC <sub>0-∞</sub> ( $\mu$ g-h <sup>2</sup> /ml)	735.1 $\pm$ 275.18	1346 $\pm$ 464.9***
MRT(h)	15.8 $\pm$ 1.255	17.43 $\pm$ 0.0051 <sup>r</sup>

Mean  $\pm$ SD (n=6);

\* Significant at P<0.005 compared to Gliclazide control.

\*\* Significant at P<0.001 compared to Gliclazide control.

\*\*\* Significant at P<0.0001 compared to Gliclazide control.

ns non-significant

Data analysis was done by using statistical Program software Prism Graph pad and the significance was determined by students paired ‘t’ test.

**CALCULATION OF BIOAVAILABILITY:**

Bioavailability is the amount of drug that available in the systemic circulation after administration of the drug. It is calculated by the following formula.

Percentage increase in Bioavailability = (AUC of test – AUC of standard)/(AUC of standard) x 100

Percentage increase in Bioavailability of Gliclazide = (79.46 – 48.63)/(48.63) x 100

Percentage increase in Bioavailability of Gliclazide = 63.39%

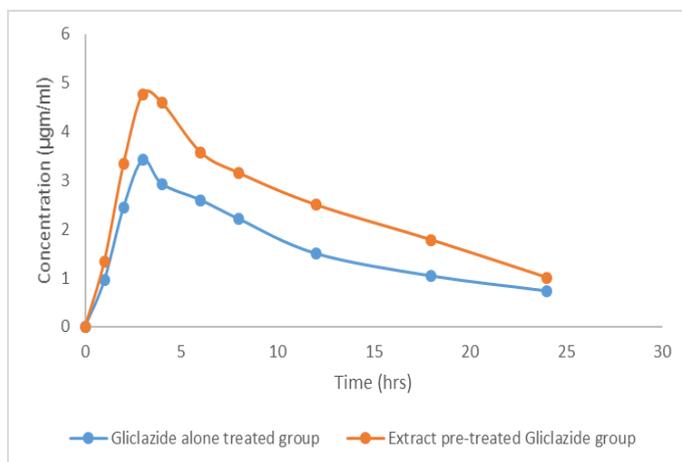


Figure 2. Mean Serum concentration time curves of Gliclazide (2 mg/kg) alone treated group and Extract (2gm/kg) pre-treated Gliclazide (2mg/kg) group.

## DISCUSSION

Drug interactions are generally evaluated in animal models. Although animal models can never replace the need for comprehensive studies in human subjects, their use can provide important insights to understand and evaluate the mechanism of potent drug interactions.

It is worth noting that several findings have confirmed the functional similarity of Cytochrome forms in rats and humans apart from convenience in serial blood sampling design suggesting that the rat is a valuable *in vivo* model for assessment of drug interactions.

Gliclazide is known to produce hypoglycaemia\ antihyperglycemic activity by pancreatic (stimulating insulin secretion by blocking K<sup>+</sup> channels in  $\beta$ -cells) and extra pancreatic (increasing tissue uptake of glucose) mechanism. Gliclazide is metabolised by CYP2C9 and CYP3A4.

*Andrographis paniculata* belongs to family Acanthaceae and its ethanolic extract is known to inhibit CYP2C9 and CYP3A4 enzymes in hepatic microsomes in human[9]. The aerial part of *Andrographis paniculata* is medicinally used for Common cold, hypertension, diabetes, cancer, malaria and snakebite, urinary tract infection[10,11].

Therefore it is necessary to adopt Therapeutic drug monitoring so as to readjust the dose and frequency of administration of Gliclazide, when they are administered concomitantly to avoid patients from severe hypoglycaemia symptoms like seizures, coma and even death.

In the present study, the blood is collected from retro-orbital plexus from both the groups of Albino wistar rats administered with Gliclazide-2mg/kg alone and Gliclazide-2mg/kg pre-treated with ethanolic extract of aerial parts of *Andrographis paniculata*-2gm/kg, centrifuged and processed with mobile phase of Phosphate buffer and acetonitrile(45:55) and injected into HPLC. Gliclazide drug peak was obtained in the chromatogram at Retention time 2.996 mins. The concentration of Gliclazide in these groups were determined by comparing the areas of peak obtained with different blood samples with serum calibrators. Other pharmacokinetic parameters were calculated by Method of residuals and AUC is calculated by trapezoidal rule. It was observed that there was a significant increase in Gliclazide C<sub>max</sub>, K<sub>el</sub>, t<sub>1/2</sub>, AUC and AUMC in the group pre-treated with Ethanolic extract of *Andrographis paniculata* when compared to Gliclazide alone treated group.

The increase in K<sub>el</sub> and t<sub>1/2</sub> indicates the interaction might have occurred in metabolism or excretion process.

The percentage increase in bioavailability of Gliclazide in extract pre-treated group was found to be 63.39 % which may cause sufficient decrease in blood glucose levels .the drug interaction may thus be due to inhibition of CYP2C9/ CYP3A4 mainly by the diterpenoids-Andrographolide content of *Andrographis paniculata* ,which leads to increase the serum levels of Gliclazide and raises serious concern about the drug safety mainly the Hypoglycemia.

Thus dose adjustment strategies are to be adopted when using Gliclazide in the Patients using herbal medicines like *Andrographis paniculata* to minimize the adverse effects.

## CONCLUSION

The extract modestly increased the bioavailability of Gliclazide. The mechanism that underlies the interaction between extract and Gliclazide involves the inhibition of CYP2C9 and CYP3A4 enzyme catalysed Gliclazide metabolism by extract.

Concomitant administration of extract could thus result in increased serum concentration of Gliclazide with increased efficacy and \or adverse effects. Thus it is necessary to adjust the dose of Gliclazide when it is administered with *Andrographis paniculata* to minimize the adverse effects of Gliclazide.

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### Cite this article as:

Mohammed Gayasuddin Mouid, Shakil Sait.S, Kavimani.S. Effect of Ethanolic Extract of Aerial Parts of *Andrographis paniculata* on the Pharmacokinetics of Gliclazide in Rats. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 5(51), 2015, 21-24.