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Original Research Article

Synergistic effect of *Allium sativum* and gliclazide on pharmacokinetic parameters in rabbit models

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Abstract

Purpose: *To assess gliclazide hypoglycemic effect in the presence of Allium sativum and determine the possible pharmacokinetics interactions of gliclazide and Allium sativa extract.*
Methods: *A set of 6 Wistar rabbits weighing between 1.35 kg - 1.75 kg was used. Gliclazide (3.7 mg/kg) was administered orally. After a washout period of 1 week, the same group of animals received Allium sativum extract (56 mg/kg) with the required quantity of water. After a further washout period of 1 week, the same group received Allium sativum (56 mg/kg) 30 min prior to the administration of gliclazide (3.7 mg/kg). Blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h and were analyzed for blood glucose using the glucose oxidase (GO)/peroxidase (PO) method. The mean significance of the pharmacokinetic parameters such as area under the curve $(AUC)_{0–24}$, biological half-life ($t_{1/2}$), maximum peak plasma concentration (C_{max}), maximum time to reach C_{max} (T_{max}), and elimination rate constant (KE) of gliclazide was estimated with and without Allium sativum in rabbits.*
Results: *Allium sativum significantly increased AUC_{0-24}, AUC_{0-a} and $AUMC_{0-24}$ and C_{max} of gliclazide ($p < 0.05$). The results revealed that Allium sativum significantly enhanced the hypoglycemic effect of gliclazide ($p < 0.05$).*
Conclusion: *Allium sativum significantly enhances the hypoglycemic effect of gliclazide by improving pharmacokinetic parameters.*

Keywords: *Allium sativum, Gliclazide, Hypoglycemic, Pharmacokinetics*

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INTRODUCTION

Many helpful medications that not only save and extend lives but also enhance our quality of life have been made possible by modern medicine. To be safe and effective, however, medications must be taken as directed. The substances in our food can have positive effects on medications [1-2]. Several dietary components and phytochemicals play significant roles in determining how drugs behave. Drug-metabolizing enzymes or transporters are frequently stimulated or inhibited, which is the fundamental mechanism behind changes in drug concentration. In addition to dietary components that cause a decrease in the plasma concentration of pharmaceuticals, nutrients cause an increase in the plasma concentration via the inhibition of drug metabolism, mainly via the inhibition of (intestinal) CYP3A4 [2-3].

An explanation for many food–drug interactions

is the suppression of CYP3A4 and/or P-glycoprotein. Many substances are inhibitors of both P-glycoprotein and CYP3A4, and many medications are substrates for both proteins. As a result, higher plasma levels of one drug caused by a concurrently administered substance may be the result of a dual effect on drug transport and metabolism. Since the main drug-metabolizing enzymes are found in the mucosa of the gut wall, it has become more evident that enterocyte metabolism can be a significant factor in the low or variable oral bioavailability of medications. Herb drug interactions can be either antagonistic or synergistic because certain components of herbs may interact with the same drug target molecules (such as enzymes or receptors). Thus, tracking medication therapy and researching drug-food interactions are needed [3-4].

Certain conditions, such as diabetes and hypertension, call for monitoring of blood pressure and blood glucose levels, respectively. Patients suffering from diabetes consume a variety of foods may lead to changes in blood glucose levels due to food–drug interactions. Therefore, drug-drug interactions may cause blood glucose levels to rise or fall. These effects can also be observed in other conditions, such as heart problems and hypertension. Drug monitoring is also necessary for medications with a low therapeutic index, such as digoxin [4-5].

Diabetes type II is more prevalent than diabetes type I (juvenile). Sulphonylureas are the medicine of choice for treating diabetes. Gliclazide is a widely used drug because of its potency, duration of action, low frequency of adverse effects, and antioxidant function. The consumption of fast food is increasing in the modern era. There are many spices in fast food, many of which are known to provide a variety of biological benefits. *Allium sativum* or Garlic is a spice that is frequently used in many Indian, Arabic, and Western cuisines. In animal models, garlic has been shown to reduce blood glucose levels. Therefore, the goal of the current study was to determine its impact on blood glucose levels in addition to gliclazide pharmacokinetic (PK) activity in healthy rabbit models [6-7].

EXPERIMENTAL

Materials

Gliclazide was obtained from Aurangabad, India. Acetonitrile was manufactured by Qualigens Chemicals, Mumbai, India. Sodium hydroxide and triethylamine were purchased from Fine Chemicals, Mumbai, India.

Animals

The procedure for determining kinetic activity was carried out as per the guidelines of the CPCSEA. The study protocol was approved by the Institutional Animal Ethics Committee, A.U. College of Pharmaceutical Sciences, Andhra University (Reg no. 634/01/a/CPCSEA), Andhra Pradesh, India and was carried out following the guidelines in the care and use of laboratory animals.

Animal studies

A set of six Wistar rabbits of both sexes, weighing between 1.35 and 1.75 kg, were in good condition. Bunnies that were in good health were maintained on a consistent diet at room temperature with a 12-hour light and dark cycle. Their enclosures were made of metal. Water was provided freely to the rabbits along with a typical animal pellet diet. A newborn oral feeding tube was inserted into the gastrointestinal tract (GIT) after the rabbit was placed on a holder, and a mouth gauge was placed between its jaws. The feeding tube was inserted into the mouth and care was taken while inserting the tube so that it does not reach the trachea [8,9]. The rabbits were given water on demand during an eighteen-hour fast before the experiment began. Additionally, water was removed during the experiment. Gliclazide therapeutic dose (TD) was orally administered to each rabbit. The identical group of animals received oral *Allium sativum* (56 mg/kg) following a week washout period. The same group was given *Allium sativum* (56 mg/kg) orally 30 min before gliclazide (3.7 mg/kg) followed by another washout period of one week. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h from marginal ear vein punctures, and blood glucose levels were measured via the glucose oxidase (GO)/peroxidase (PO) method [9-11]. Calculation of blood glucose level (G) was determined by using Eq 1.

$$G = (A_{test} - A_{blank}/A_{standard} - A_{blank})C \quad \ldots\ldots\ldots (1)$$

Where A_{test} is absorbance of test sample, $A_{standard}$ is absorbance of standard sample A_{blank} is absorbance of blank plasma sample and C is the concentration of standard plasma sample.

Assessment of pharmacokinetic parameters

Non-compartmental analysis of plasma data after extravascular input was evaluated using PK Solver 2.0 (MS-Excel add-in). The pharmacokinetic parameters; area under the

curve (AUC_{0-t} $AUC_{0-\infty}$), Elimination rate constant (K_{el}), elimination half-life ($t_{1/2}$), area under the first moment of plasma concentration-time curve (AUMC) and Mean residence time (MRT_{0-24}) were calculated. All the pharmacokinetic parameters were expressed as mean ± standard deviation (SD).

Statistical analysis

Statistical analysis was performed using Prism 5.0 software trial version (Graph pad Inc. USA). ANOVA was used for comparison of the pharmacokinetic parameters. All the statistical tests were performed at a significance level of 0.05 [12].

RESULTS

The PK parameters and hypoglycemic activity for gliclazide and gliclazide with *Allium sativum* were estimated separately. In gliclazide-treated matching control group, the maximum percentage decrease in blood glucose decreased, and the peak serum gliclazide concentrations were 34.9 % and 384 ng/mL at 3 h, respectively. When gliclazide was given for drug delivery, the percentage decrease in blood glucose was 44.16 ± 0.9 at 3 h, and the peak serum gliclazide concentration was 451.4 ± 0.1 ng/mL at the same time. The hypoglycemic effect of various doses of gliclazide has been shown in (Figure 1). Based on the graphical representation, gliclazide at 2TD was optimized to incorporate *Allium sativum* further to improve the PKs of gliclazide which has been shown in (Figure 2). The serum gliclazide concentrations with (3.7 mg/kg) and without *Allium sativum* (104 mg/kg) in rabbits are shown in (Figure 3), and the hypoglycemic activity of the drug improved in the presence of *Allium sativum.* The PK parameters of gliclazide with (3.7 mg/kg) and without *Allium sativum* treatment (104 mg/kg) are shown in (Table 1).

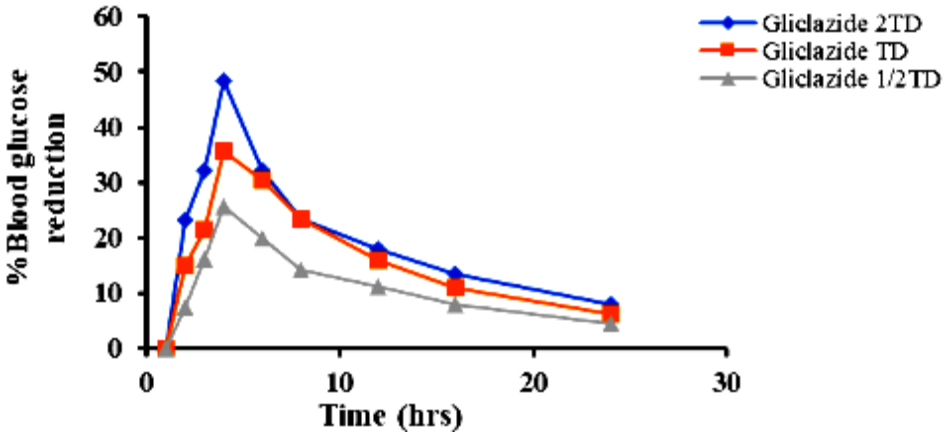

Figure 1: Percent blood glucose reduction of gliclazide in Normal rabbits (n=6)

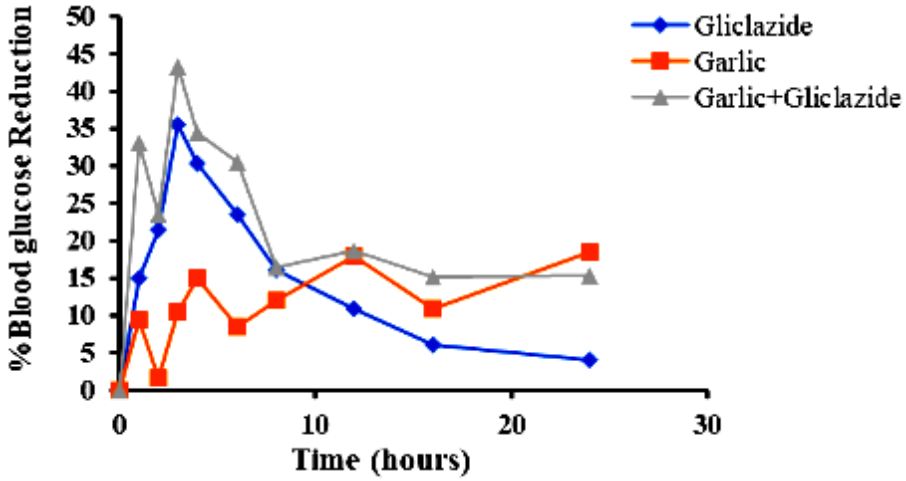

Figure 2: Effect of *Allium sativum* on the hypoglycaemic activity of gliclazide in rabbits (n=6)

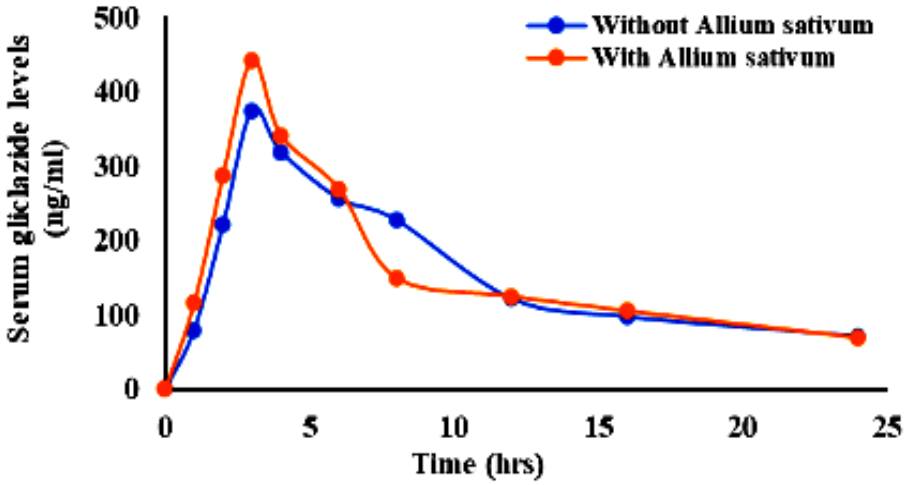

Figure 3: Serum concentration levels of gliclazide with and without *Allium sativum*

Table 1: Pharmacokinetic parameters of gliclazide with and without *Allium sativum*

Pharmacokinetic parameter	Without *Allium sativum*	With *Allium sativum*	*P* < 0.05
AUC0-24	3188.99±233.29	3758.562±108.824*	Significant
AUC0-a	3840.23±231.90	4596.94±210.45*	Significant
AUMC0-24	35538.51±991.84	42889.56±2706.5*	Significant
AUMC0-a	59008.14±3270.31	73263.63±7246.2	Not significant
Ke	0.0911±0.00603	0.0838±0.003167	Not significant
Ka	1.53	1.15	Not significant
T1/2	8.23±0.293	8.22±0.37	Not significant
Vdss	16.73±3.93	15.65±0.61	Not significant
Cl	1080.94±145.57	1077.23±67	Not significant
Cmax	346.74±3.41	441.88±11.42***	Significant
Tmax	3.00	3	Not significant
MRT	15.67±1.55	15.76±0.8	Not significant

Note: *P < 0.05, ***p < 0.001 vs gliclazide

Pharmacokinetic data generated were subjected to Student *t*-test to obtain mean PKs. Significant changes were observed statistically in parameters such as AUC $_{0\text{-}t\ (24)}$, AUC $_{0\text{-}\infty}$, AUMC, C_{max}, Ke and elimination $t_{½}$, respectively. The AUC and AUMC of gliclazide were significantly altered in the combination group from 3188.99 ng/mL(h) and 35538.51 ng/mL(h^2) to 3758.56 ng/mL(h) and 42889.56 ng/mL(h^2), respectively, compared with those of gliclazide-treated group. The C_{max} significantly increased from 346.74 to 441.88 ng/mL, indicating that the activity of gliclazide may change *Allium sativum*. The Vd was nearly the same at 16.73 and 15.65 L. The T_{max} remained unchanged. The absorption half-life ($t_{1/2}$(a)) and absorption rate constant (Ka) remained unchanged, indicating that the absorption was not altered. The elimination half-life ($t_{1/2}$) and elimination rate constant (Kel) were significantly altered or excreted from gliclazide in the presence of *Allium sativum*. The mean residence times (MRTs) of gliclazide before and after treatment were 15.67 and 15.76 h, respectively.

DISCUSSION

When the regular dosage of the drug was reduced, PK parameters such as $AUC_{0–24}$, $t_{1/2}$, C_{max}, T_{max}, and K_e were shown to have the highest values. From the results of blood glucose, it was indicated that *Allium sativum* has no discernible effect on blood glucose levels but when the drug was given along with *Allium sativum*, serum concentration levels were improved due to high hypoglycemic activity of *Allium sativum.* Results from a previous study have shown that *Allium sativum* amplifies the hypoglycemic effect of gliclazide [13].

Various doses of gliclazide have been shown to have hypoglycemic effects on the serum of rabbits, as indicated by the observation of the highest peak due to *Allium sativum.* The various doses of gliclazide resulted in the highest peak plasma concentrations. *Allium sativum* enhanced the hypoglycemic effect of gliclazide and resulted in the highest peak which is well correlated with serum levels of gliclazide. Therefore, *Allium sativum* effectively decreased blood glucose

levels, resulting in a rise in drug concentration in serum at the same time. The result may be due to the combinational hypoglycemic effect of both gliclazide and *Allium sativum* [14].

Pharmacokinetic parameters were observed in rabbits treated with and without *Allium sativum*. The increases in AUC, AUMC, Ke, and $t_{1/2}$ indicate that there is an interaction during the metabolism or excretion of gliclazide [15]. Since there is no change in clearance, the interaction may not be at the excretion level. The active constituent of *Allium sativum, S-allyl cysteine,* was reported to inhibit CYP 2C9 enzyme in rat model. The same enzyme is also responsible for the partial metabolism of gliclazide. Hence, increase in serum levels and changes in PK parameters of gliclazide in the presence of *Allium sativum*.

Allium sativum increased the mean percent blood glucose reduction of gliclazide in rats; this interaction may be due to either its PK or pharmacodynamic nature. The overall serum gliclazide levels increased from 1–24 h, and there were significant changes in the C_{max}, AUC $_{(0–24)}$, AUC $_{(0–\alpha)}$, AUMC $_{(0–24)}$, Kel and $t_{1/2}$. The result findings indicated that *Allium sativum* increases the bioavailability of gliclazide. These results indicate that the interaction may occur during the metabolism/excretion phases. Since there was no change in clearance, the possible route of interaction may be metabolism. The possible mechanism of action of the interaction at the site of metabolism may involve s-ally cysteine, which is one of the main active constituents of garlic and has the capacity to inhibit the hepatic microsomal enzyme (CYP P450 2C9), which is also responsible for the metabolism of gliclazide [16].

CONCLUSION

Allium sativum improves hypoglycemic effect and pharmacokinetic parameters of gliclazide in rabbit model. Gliclazide at various doses had a dose-related hypoglycemic effect on rats, and blood glucose levels changed with *Allium sativum*. Nevertheless, when it is used in combination with gliclazide, an increased hypoglycemic effect is observed in healthy rats. Hence, demonstrating interaction between gliclazide and *Allium sativum*.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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