Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 871 - 876

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INFLUENCE OF GARLIC OIL ON ANTI DIABETIC ACTIVITY OF GLICLAZIDE IN DIABETIC RATS

SRINIVAS NANDYALA*, G. VIJAYA KUMAR, M SRINIVASU AND B SRAVANI

Department of Pharmacology, A.K.R.G College of Pharmacy, Nallajerla, Andhra Pradesh, India.

ABSTRACT

The objective of the present study is to examine the influence of garlic oil on the hypoglycaemic activity of gliclazide in alloxan induced diabetic rats. Albino rats were divided into five groups of six each. Group I animals served as normal control, group II animals served as diabetic control, group III animals treated with gliclazide, group IV animals treated with garlic oil and group V animals received both gliclazide and garlic oil. Blood samples were collected from rats and were analyzed blood glucose levels by GOD/POD method. Combination of gliclazide and garlic oil treated group showed significant reduction in percentage of blood glucose levels when compared to gliclazide only treated group. The present study suggests that during the simultaneous administration of gliclazide and garlic oil the dose and frequency of administration of gliclazide has to be readjusted accordingly in order to avoid severe hypoglycaemic complication due to the drug-drug interactions.

KEYWORDS: **Garlic oil, gliclazide, hypoglycaemic activity, alloxan.**

SRINIVAS NANDYALA
Department of Pharmacology, A.K.R.G College of Pharmacy, Nallajerla, Andhra Pradesh, India.

***Corresponding author**

INTRODUCTION

A drug interaction is the quantitative or qualitative modification of the effect of a drug by the simultaneous administration of different drugs[1]. When discussing drug interactions, the drug affected by the interaction is called the "object drug" and the drug causing the interaction is called the "precipitant drug." It is order of the day to prescribe more than one drug. If two drugs are given together their combined effects may be greater, same as or smaller than the sum of the effects of the individual drugs or no effect at all. The three possible effects are known as potentiation, addition (or summation) and antagonism respectively[2]. In case of the associated diseases in diabetes, the incidence of fungal infections, cardiovascular disorders, nephropathy, retinopathy, neuropathy, sexual impotence, hyperacidity and respiratory tract infections are quite high[3]. Multi-drug therapy is commonly followed which may often lead to deleterious and hazardous drug interactions. There is every possibility of administration of other drugs along with sulphonylureas in treating type-II diabetes which create drug interaction problems.

Gliclazide is a second generation sulphonylurea oral hypoglycaemic agent used in the treatment of non-insulin-dependent diabetes mellitus. It improves defective insulin secretion and may reverse insulin resistance observed in patients with non-insulin-dependent diabetes mellitus. These actions are reflected in a reduction in blood glucose levels which is maintained during both short and long term administration, and is comparable with that achieved by other sulphonylurea agents. Gliclazide binds to the β cell sulfonyl urea receptor (SUR1). This binding subsequently blocks the ATP sensitive potassium channels. The binding results in closure of the channels and leads to a resulting decrease in potassium efflux leads to depolarization of the β cells. This opens voltage-dependent calcium channels in the β cell resulting in calmodulin activation, which in turn leads to exocytosis of insulin containing secretory granules[4]. Gliclazide extensively metabolized in the liver by Cytochrome P450 2C9 and Cytochrome P450 2C19[5].

Garlic (*Allium sativum*) has been used medicinally, and as a culinary ingredient, for over 5000 years. The Greeks also used it extensively: Hippocrates recommended it for infections, wounds, cancer and leprosy; Dioscides for heart problems, and Pliny listed it in 61 remedies for a variety of ailments. Currently in the US and Western Europe, garlic is one of the most popular substances used to reduce various risks associated with heart disease. Most of garlic's popularity is based on the herb's well-known folk uses and scientific research on the benefits of garlic for heart health. These health promoting benefits may be experienced by using garlic as both a food ingredient and a dietary supplement. Garlic is odiferous, tasty, and medicinal. The first medical textbook known to have discussed the use of garlic in medicine was the Collection of Commentaries on the Classic of the Materia Medica, written over 1,500 years ago. Extracts of fresh garlic and garlic oil exhibited an inhibitory effect on cytochrome P450 2C9, 2C19, 3A4, 3A5 and 3A7 mediated metabolism of a marker substrate[6]. The present study has been undertaken to evaluate the influence of garlic oil on the pharmacodynamics of gliclazide. In this study the influence of garlic oil on the hypoglycaemic activity of Gliclazide was evaluated by compare the percentage blood glucose reduction in alloxan induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Reagents

Gliclazide was gift sample from Wockhardt Ltd., Mumbai, India. Garlic pearls were purchased from Ranbaxy Laboratories Limited, Mumbai, India. Alloxan and Dichloromethane were purchased from Loba chemie Pvt. Ltd., Mumbai,

India. The other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats weighing 180–200g of either sex were obtained from the animal house of A.K.R.G. College of Pharmacy, Nallajerla, Andhra Pradesh, were used for this study. The animals were housed in separate groups (six rats in each cage) in clean sanitized polypropylene cages containing sterile paddy husk as bedding. The bedding material of the cages was changed every day. They had free accessed to standard pellet diet and water *ad libitum*. The animals were maintained under day and night 12:12 hrs cycles and with maintenance of room temperature 25 ± 2°C. All procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under ministry of Animal Welfare Division, government of India, New Delhi. IAEC approved the experimental protocol (AKRGCP/IAEC/01/2011-12) dated 11/02/2012.

Induction of diabetes mellitus

Wistar albino rats of either sex weighing between 180-200 g were selected and fasted for 18 hrs and provided sufficient water *ad-libitium*. The animals were randomly distributed into different groups. The animals were kept in colony cages at standard husbandry conditions. Diabetes was induced in rats by the administration of alloxan monohydrate in two doses, i.e. 100 mg/kg and 50 mg/kg body weight intraperitoneally for two consecutive days[7]. After 72 hrs, samples were collected from rats by orbital puncture of all surviving rats and plasma was analysed for glucose levels. Rats which have shown more than 200 mg/dl blood glucose levels were considered as diabetic. The blood glucose levels were monitored for further three days. From this it was confirmed that diabetes was induced in 24 hrs and stabilized within 3 days. These animals were used for further studies.

Collection of blood samples

Blood samples were withdrawn from retro orbital plexus[8]. These blood samples were analysed for blood glucose by GOD/POD method[9].

Influence of garlic oil on the hypoglycaemic activity of gliclazide in rats[10, 11]

Wistar albino rats of either sex weighing between 180-200 g were selected and divided into five groups of six animals each. The treatment schedule was as follows:

Group I : Vehicle (Normal control)
Group II : Alloxan (Diabetic control)
Group III : Alloxan + Gliclazide (2mg/kg., p.o.)
Group IV : Alloxan + Garlic oil (1ml/kg., p.o.)
Group V : Alloxan + Garlic oil (1ml/kg., p.o.) + Gliclazide (2mg/kg., p.o.)

The study was divided into 3 phases. In the first phase, garlic oil (1ml/kg., p.o.) was administered to group IV and group V, and vehicle was administered to group I, group II and group III continuously for 14 days. In the second phase, on the 15th day diabetes induced by the administration of alloxan monohydrate in two doses, i.e. 100 mg/kg and 50 mg/kg body weight intraperitoneally for two consecutive days to all the groups of animal except group I which is treated as normal control. In the third phase, on the 17th day vehicle administered to group I and group II, gliclazide (2mg/kg., p.o.) administered to group III, garlic oil (1ml/kg., p.o.) administered to group IV, and garlic oil (1ml/kg., p.o.) administered to group V 30 minutes prior to the administration of Gliclazide (2mg/kg., p.o.). Blood samples were collected thereafter at different time intervals up to 12 hours and were analyzed by GOD/POD method. Blood glucose levels were expressed as mg/dL of blood. The percentage reduction in blood glucose levels at time‘t’ was calculated by using the following formula.

$$\text{Percentage blood glucose reduction at time't'} = \frac{A-B}{A} \times 100$$

Where, A = Initial blood glucose level before drug administration.
B = Blood glucose levels at time't' after the drug administration.

Statistical analysis[12]

The data are expressed as mean ± S.E.M. The data was evaluated statistically by using one way ANOVA followed by student t-test. The results were considered statistically significant when the $p < 0.05$.

RESULTS

The mean blood glucose levels of different groups (vehicle treated, gliclazide treated, garlic oil treated, and combination of gliclazide and garlic oil treated) were measured by the GOD/POD method and tabulated in table 1. The graphical representation was shown in graph 1. The mean percentage blood glucose reductions were calculated and tabulated in table 2. The graphical representation was shown in graph 2. The data was treated statistically and the statistical interaction implies that the difference in blood glucose levels was statistically significant between gliclazide with garlic oil and gliclazide without garlic oil treated groups.

Table 1
Mean blood glucose levels in alloxan induced diabetic rats

S. No	Time (hrs)	Blood glucose level (mg/dL) (Mean ± S.E.M.)				
		Vehicle (Normal control)	Alloxan (Diabetic control)	Alloxan + Gliclazide	Alloxan + Garlic oil	Alloxan + Garlic oil + Gliclazide
1	0	86±6.5	259±7.6	256±7.4	253±5.9	249±6.3
2	1	84±3.5	254±8.4	203±5.2*	250±6.8	184±5.7**
3	2	82±2.6	256±6.3	151±8.1*	244±7.4	143±5.4*
4	3	85±2.4	250±8.6	105±5.9*	241±8.1	102±4.2*
5	4	82±4.3	247±4.4	103±4.8*	228±7.9	89±4.4**
6	6	84±4.9	236±7.4	118±4.3*	224±8.7	86±2.1**
7	8	75±3.8	234±7.1	124±4.9*	221±2.9	82±4.6**
8	12	72±5.4	232±8.2	128±6.7*	213±6.2	83±3.7**

S.E.M. – Standard Error of Mean, * p< 0.05 when compared with diabetic control group.
***** p< 0.01 when compared with diabetic control group.***

Graph 1
Mean blood glucose level profile of alloxan induced diabetic rats

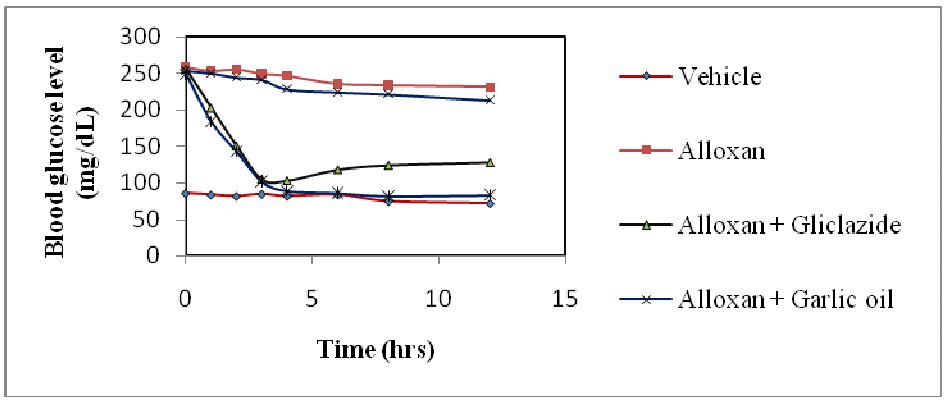
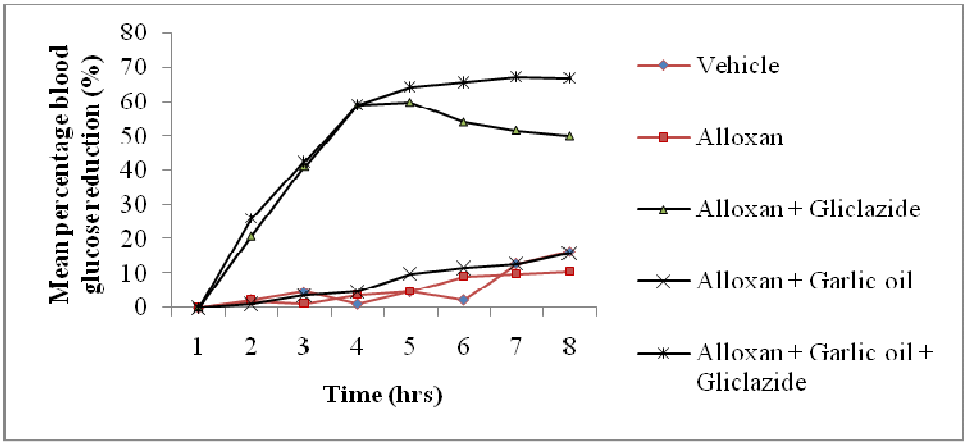


Table 2
Mean percentage blood glucose reduction in alloxan induced diabetic rats

S. No	Time (hrs)	Blood glucose level (mg/dL) (Mean ± S.E.M.)				
		Vehicle (Normal control)	Alloxan (Diabetic control)	Alloxan + Gliclazide	Alloxan + Garlic oil	Alloxan + Garlic oil + Gliclazide
1	0	0	0	0	0	0
2	1	2.3±0.15	1.9±0.12	20.7±1.30**	1.1±0.08	26.1±1.44**
3	2	4.6±0.23	1.1±0.31	41.0±1.56**	3.5±012	42.5±1.70**
4	3	1.1±0.31	3.4±0.73	58.9±1.18**	4.7±0.14	59.0±1.61**
5	4	4.6±0.24	4.6±0.25	59.7±1.65**	9.8±1.43	64.2±1.57**
6	6	2.3±0.19	8.8±0.09	53.9±1.06**	11.4±1.24	65.4±1.85**
7	8	12.7±0.58	9.6±0.51	51.5±1.62**	12.6±1.41	67.0±1.27**
8	12	16.2±0.42	10.4±0.47	50.0±1.27**	15.8±1.48	66.6±1.66**

*S.E.M. – Standard Error of Mean, * p< 0.05 when compared with diabetic control group.*
*** p< 0.01 when compared with diabetic control group.*

Graph 2
Mean percentage blood glucose reduction profile of alloxan induced diabetic rats



DISCUSSION

In this present study diabetes was induced by intra peritonial injection of alloxan prior to the study and which animals were showed more than 200 mg/dL were selected for the study. Gliclazide is a second generation sulphonylurea oral hypoglycaemic agent used in the treatment of diabetes mellitus. It binds to the β cell sulfonyl urea receptor. This binding subsequently blocks the ATP sensitive potassium channels. The binding results in closure of the channels and leads to a resulting decrease in potassium efflux leads to depolarization of the β cells. This opens voltage-dependent calcium channels in the β cell resulting in calmodulin activation, which in turn leads to exocytosis of insulin containing secretory granules[4]. Garlic has been used as prophylactic and therapeutic agent for many years for such purposes as treating tumour, wounds, and heart diseases.. Garlic pearls which are contained garlic oil are widely available as OTC product and provide a range of supplemental benefits associated with the sulfur compounds in the plant. Hence many of the people used these garlic pearls as self medication to control the blood pressure, heart diseases, and digestion problems.

Gliclazide extensively metabolized in the liver by cytochrome P450 2C9 and cytochrome P450 2C19. Extracts of fresh garlic and garlic oil exhibited an inhibitory effect on cytochrome P450 2C9, 2C19, 3A4, 3A5 and 3A7 mediated metabolism of a marker substrate. Based on

these two keypoints in the present study an attempt was made to evaluate the effect of garlic oil on the hypoglycaemic activity of gliclazide. The data was treated statistically and the statistical interaction implies that the difference in blood glucose levels was statistically significant between gliclazide with garlic oil and gliclazide without garlic oil treated groups. From the results it was revealed that the highest percentage blood glucose level reduction was observed in gliclazide with garlic oil treated group (67.0±1.27% at 8th hour) when compared to gliclazide without garlic oil treated group (51.5±1.62% at 8th hour). It may be due to the garlic oil has been exhibited inhibitory effect on cytochrome P450 2C9, 2C19 mediated metabolism of gliclazide, leads to potentiation and prolongation of anti hyperglycemic activity of gliclazide.

CONCLUSION

We wanted to evaluate the influence of garlic oil on the pharmacodynamics of gliclazide. From the present study it has been concluded that the garlic oil potentiated anti hyperglycemic activity of gliclazide due to the inhibitory effect on cytochrome P450 2C9, 2C19 mediated metabolism of gliclazide. Thus the present study suggests that during the simultaneous administration of gliclazide and garlic oil the dose and frequency of administration of gliclazide has to be readjusted accordingly in order to avoid severe hypoglycaemic complication due to the drug-drug interactions. However the present study warrants further studies to find out the relevance of the interaction in human beings.

REFERENCES

1. Morales Olivas FJ and Estan L, Anti hypertensive drug-drug interactions. Med Clin (Barc), 124 (20): 782-789, (2005).
2. James Crossland, Lewis's Pharmacology, 4th Edn, E and S Livingstone (London): 21-22, (1970)
3. S. Satyanarayana, Y. S. R. Krishnaiah, K. Eswar Kumar, I. Elisha Raj and V.V.S. Kanti Kiran, Influence of quinidine, selegine and amphotericin-B on the pharmacokinetics and pharmacodynamics of tolbutamide in rabbits. Indian Drugs, 35 (10): 1-8, (1998).
4. Hoich RI, Insulin potentiating action of gliclazide. Pharmacol Res Commun, 18(5): 419-430, (1986).
5. Ananya Sarkar, Ajay Tiwari, Parminder S. Bhasin and Moloy Mitra, Pharmacological and Pharmaceutical Profile of Gliclazide. Journal of Applied Pharmaceutical Science, 01 (09): 11-19, (2011).
6. Foster BC, Foster MS, Vandenhoek S, Krantis A, Budzinski JW, Arnason JT, Gallicano KD, and Choudri S, An in vitro evaluation of human cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. J Pharm Pharm Sci, 4 (2): 176-84, (2001)
7. K. Eswar Kumar, P. D. Jyotsna Rani, K. Raghu Ram, P. Swathi and M. N. Gupta, Pharmacodynamic and pharmacokinetic drug interaction of gliclazide and risperidone in animal models. Int J Pharm Pharm Sci, 4 (2): 659-660, (2012).
8. Riley V, Adaptation of orbital bleeding technique to rapid serial blood studies. Proc. Soc. Exp. Biol. Med., 104: 751-754, (1960).
9. Trinder P, Determination of blood glucose using an oxidaase-peroxidase system with a non carcinogenic chemogen. J Clin Pathol, 22: 158-161, (1969).
10. S K Mastan and K Eswar Kumar, Influence of indinavir on the pharmacodynamic activity of gliclazide in rats and rabbits. IJIB, 7 (1): 16-21, (2009).
11. Saswata Banerjee, Hariom Singh and Tapan Kumar Chatterjee, Evaluation of Anti Diabetic and Anti Hyperlipidemic Potential of Methanolic Extract of *Juniperus Communis* (L.) in Streptozotocinnicotinamide Induced Diabetic Rats. Int J Pharm Bio Sci, 4(3): 10-17, (2013).
12. Satyajit Mohapatra, Supriya Pradhan, Bandana Rath and Saswati Tripathy, Antioxidant Properties of Aloe Vera in Streptozotocin Induced Diabetic Rats. Int J Pharm Bio Sci, 4(3): 187-191, (2013).

Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 871 - 876