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HERB DRUG INTERACTIONS OF NEEM LEAF EXTRACT WITH GLIMEPIRIDE IN DIABETIC RATS

G. Sumalatha[1], M. Vidyavathi[*,2] and G. Thirupathi [3]

[1]MAX institute of Pharmaceutical Sciences, Khammam, Andhra Pradesh, India

[*, 2] Institute of Pharmaceutical Technology, SPMVV, Tirupati, Andhra Pradesh, India

[3]Vaagdevi Pharmacy College, Bollikunta, Warangal, Telangana State, India

***Corresponding Author Email: vidyasur@rediffmail.com**

ABSTRACT

Herb Drug interaction is a challenging concept, since the consumption of herbal and other drugs is not documented in patient's profile. With this aspect the present study was designed to investigate the pharmacokinetic and Pharmacodynamic effect of neem leaf extract (NE) on the pharmacokinetics and pharmacodynamics of Glimepiride in diabetic rats. All the pharmacokinetic parameters such as C_{max}, AUC_{0-n}, AUC_{tot}, $t_{1/2}$ are increased and clearance and V_d are decreased when compared with control group. In Pharmacodynamic study the blood glucose levels are estimated, total antioxidant status in diabetic rats by using DPPH method and oral glucose tolerance test were performed. Combination has improved the total antioxidant status. The results revealed that combination of Glimepiride with neem leaf extract leads to enhancement of bioavailability of Glinepiride, this suggest that neem leaf extract might be beneficial as an adjuvant to Glimepiride in diabetic patients.

KEY WORDS

Neem leaf extract, Glimepiride, Pharmacokinetics, Pharmacodynamics.

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INTRODUCTION:

Diabetes Mellitus (DM) is a common metabolic disorder characterized by hyperglycemia, glycosuria, polymea and Polydipsia induced by insulin deficiency[1] and insulin resistance[2]. Recent estimates indicate that there were 171 million people in world with Diabetes in the year 2000 and this may be projected to increase to 366 million by 2030[2]. Combination of herbal drugs is found to be beneficial in certain diseases when given along with conventional drugs.

Azadirachta indica (neem) belonging to *Meliaceae* family is very important medicinal plant which is traditionally used to treat different diseases. It is native to India, Bangladesh, Thailand, Nepal & Pakistan. It is growing well in tropical and sub-tropical regions. Neem is most important medicinal plant that has been declared worldwide as the "Tree of the 21st century" by the United Nations. In India, it is called "Divine Tree" ,"Life giving tree", "Nature's Drugstore", "Village Pharmacy" and "Panacea for all diseases" [3,4,5,6]. The chemical constituents are found in the leaves of neem as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and aminoacid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol[7,8,9] . Traditionally, neem is also widely used in Indian Ayurvedic medicine system for the treatment of chronic disease like diabetes [8,10,11].

The aim of the present study was to investigate the effect of neem leaf extract on the pharmacokinetics and pharmacodynamics of glimepiride in streptozotocin induced diabetic rats. There are several study reports of NE on inhibition of microsomal enzyme system (CYP1A1, 1A2 and 3A4) and may lead to change in the

bioavailability of concomitant drugs[16]. Hence, there is the possibility of NE for the metabolic inhibition of Glimepiride.

MATERIALS AND METHODS:

Glimepiride was obtained from Dr. Reddy's laboratories (Hyderabad, India). Methanol (HPLC-grade), potassium dihydrogen orthophosphate and orthophosphoric acid of AR grade (99.5%) were procured from Merck Specialties Pvt. Ltd., Mumbai. Ascorbic acid, α, α-diphenyl-β-picrylhydrazyl (DPPH), and streptozotocin (STZ) were purchased from Hi Media Laboratories Pvt. Ltd, Mumbai. Water for analytical purpose is double distilled, filtered by using direct-Quv millipore and sonicated for removing air bubbles. All other chemicals used were of analytical grade.

Maintenance of animals

Male Albino Wistar rats weighing 180-250 g were purchased from Mahaveer enterprises, Hyderabad, India. The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycle; at an ambient temperature of 25 ± 5°C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water ad libitum. All experimental protocols were approved by the Institutional animal ethical committee(1533/PO/a/11/CPCSEA).

Pharmacokinetic and Pharmacodynamic study in diabetic rats:

Induction of diabetes in rats:

Diabetes was induced by using streptozotocin (55 mg/kg, b.w., i.p.) in citrate buffer (pH 4.5) to the overnight fasted Wistar rats[15]. After 72 h, blood samples were collected from rats by retro orbital puncture and the serum was analyzed for glucose levels. Animals with blood glucose level >250 mg/dl were considered as diabetic and were used for the study.

Grouping of diabetic rats and treatment:

Diabetic rats were divided into 4 groups Group-I rats were treated as diabetic control which are treated with normal saline

Group-II rats were administered with Glimepiride (1 mg/Kg P.O.) on 8th day.

Group- III rats were treated with Ethanolic neem leaf extract (200 mg/Kg) for 8 days.

Group- IV rats were pre-treated with Ethanolic neem leaf extract for 7 days and on 8th day with Glimepiride (1 mg/Kg) followed by Ethanolic neem leaf extract.

Before the collection of blood samples animals were fasted for 16 h with water ad libitum. Blood samples were collected from retro-orbital vein puncture [14] using heparinised capillary tubes at 0, 2, 4, 6, 8, 12 and 24 h. Serum was separated after centrifugation at 8000 rpm for 15 min and used for further analysis.

HPLC analysis of glimepiride in diabetic pretreated rats: -

Serum glimepiride concentration was determined by reverse phase HPLC[12]. Briefly, the solvent delivery system was a shimadzu pump model LC – 20 AD (Japan) and the column used was Lichrosphere 100 RP C_{18} column effluent was monitored with SPD-M10Avp diode array detector at 230 nm. The HPLC system was equilibrated with the mobile phase consisting of methanol: 10mm potassium dihydrogen ortho phosphate (pH 3.0) , (80:20 v/v), at a flow rate of 1.0 ml/min. Serum samples were denatured by methanol and then centrifuged at 8000 rpm for 15 min. 20 µl of clear supernatant was injected into the HPLC system for quantification.

Pharmacodynamic Study: -

The blood samples were analysed for blood glucose using glucose oxidase-peroxidase method[13].

Oral glucose tolerance test (OGTT) in STZ- induced diabetic rats.

The diabetic overnight fasted rats were divided into 4 groups (n=6) and treated same as mentioned above study. The rats of all the groups were loaded with D-glucose (2 g/Kg) 30 min after the treatment. Blood samples were collected from the rats at 30, 60, 90 & 120 min after glucose loading for determination of blood glucose levels[14].

Estimation of total antioxidant status in diabetic pretreated rats: -

The serum samples of sub-acute study were used to determine the total antioxidants status by using DPPH method[15]. Ascorbic acid was used as reference standard. The standard graph was prepared using different concentrations of ascorbic acid in water (y=0.0018 + 0.0116, r= 0.9953) and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Statistical analysis:

The pharmacokinetic parameters were calculated using Kinetica Software (version 4.4.1). All values of pharmacokinetic and pharmacodynamic studies were expressed as mean ± SD. The data were statistically evaluated using one-way analysis of variance (ANOVA)

followed by post hoc Dunnet's t-multiple comparison tests using Graph Pad Prism 4 computer software. Values corresponding to $p \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

The pharmacokinetic parameters of glimepiride were significantly increased in co-administration of NE as shown in Table 1, i.e. C_{max} (1.45 times), AUC_{0-n} (1.92 times), AUC_{total} (1.96 times), t1/2 (1.67 times), MRT (1.79 times), whereas the clearance (0.75 times) and volume of distribution (0.58 times) of Glimepiride was decreased, when compared with Glimepiride alone group**.** The T_{max} of Glimepiride was not altered by concurrent administration with NE.

Table 1: **Pharmacokinetic Parameters of Glimepiride in STZ induced diabetic rats**

PK parameters	Glimepiride	Glimepiride +Neem extract
C_{max} (µg/ml)	**18.23± 0.82**	**26.42± 1.46****
T_{max} (h)	**2**	**2**
AUC_{0-n} (µg/ml h)	**68.02± 2.54**	**130.64± 3.24****
AUC_{tot} (µg/ml h)	**70.62±2.01**	**138.48± 3.84****
t½ (h)	**1.96± 0.62**	**3.27± 0.82***
MRT (h)	**3.5± 0.4**	**6.26± 0.2****
tCL (ml/min)	**130.2± 1.59**	**98.2± 1.8****
Vd (ml)	**38.6± 5.29**	**22.4± 2.6****

All values are expressed as mean ± SD (n=6). *p<0.05; **p<0.01 considered as significant when compared with Glimepiride control

Definitions of the parameters:

C_{max}: Peak serum concentration; T_{max}: Time to reach peak serum concentration; AUC_{0ton}: Area under serum concentration/time plot until the last quantifiable value; AUC_{total}: Area under serum concentration/time plot extrapolated to infinity; $t_{1/2}$: Terminal half-life; MRT: Average mean residence time; CL: Total clearance; V_d: Volume of distribution

Table2: Mean serum glucose levels and percentage reduction of serum glucose levels

Group No.	Treatment	Dose (mg/Kg)	Serum glucose levels (mg/dl) and % reduction of serum glucose levels at different hours						
			0 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr
I	Control	-	334.56±2.4	332.58± 1.54 (0.49%)	332.42± 1.8 (0.65%)	331.68± 1.46 (0.85%)	332.2± 1.2 (0.53%)	332.8± 2.0 (0.54%)	332.2± 1.45 (0.39%)
II	Glimepiride	1 mg/kg	340.5± 4.6	276.5± 5.9 (19.81%)**	220.46± 9.4 (29.14%)**	175.05± 6.8 (50.63%)**	233.15± 8.6 (32.58%)**	282.3± 6.8 (17.1%)**	332.27± 2.4 (3.46%)*
III	Neemleaf extract	200 mg/kg	229.6± 5.6	199.8± 6.8 (14.1%)**	178.3± 4.9 (23.3%)**	136.2± 6.8 (41.72%)**	122.01± 4.5 (46.8%)**	156.8± 6.6 (31.1%)**	184.0± 8.4 (19.7%)**
IV	NE+ Glimepiride	200 mg/kg + 1 mg/kg	358.2± 6.3	396.14± 8.5 (18.4%)**	237.6± 9.4 (34.8%)**	168.9± 7.7 (52.81%)**	219.8± 8.9 (39.9%)**	270.4± 9.2 (24.6%)**	296.6± 6.4 (17.4%)**

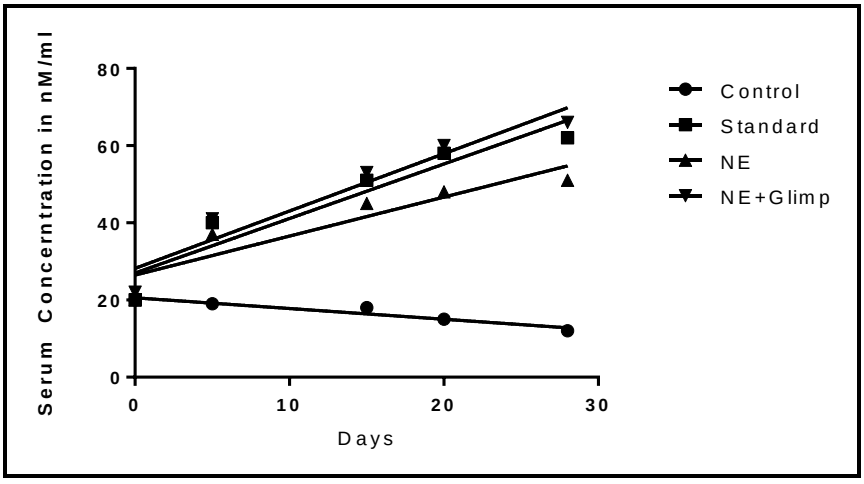
All values are expressed as mean ± SD (n=6);*P < 0.05, **P< 0.01 considered as significant when compared with Control.

Table 3: Oral glucose tolerance and percentage reduction of serum glucose levels in STZ induced diabetic rats

Group No.	Treatment	Dose (mg/Kg)	Blood glucose levels (mg/dl) at different time intervals				
			0 Min	30 Min	60 Min	90 Min	120 Min
I	Control	-	350.32± 2.94	346.25± 2.94 (2.2%)	334.28± 3.64 (5.68%)	319.14± 2.65 (8.99%)	306.6± 2.64 (12.68%)
II	Glimepiride	1 mg/kg	386.52± 3.24	346.8± 3.01 (10.46%)**	319.52± 2.96 (17.4%)**	260.6± 2.7 (33.7%)**	232.32± 3.14 (39.9%)**
III	NE	200 mg/kg	372.14± 4.6	348.4± 2.82 (7.56%)**	303.0± 2.46 (18.65%)**	286.4± 4.2 (24.2%)**	258.4± 2.13 (31.6%)**
IV	NE+Glimepiride	200 mg/kg + 1 mg/kg	392.6± 2.6	352.3± 4.8 (11.2%)**	306.4± 3.24 (22.04%)**	264.4± 5.2 (33.6%)**	232.4± 4.8 (41.8%)**

All values are expressed as mean ± SD(n=6);*p<0.05; **p<0.01 considered as significant when compared with control.

Fig 1: Effect of sub-acute pretreatment of NE and Glimepiride on total antioxidant status of STZ-induced diabetic rats



The significant increase in pharmacokinetic parameters such as C_{max}, AUC_{0-n}, AUC_{total}, t1/2 and MRT was observed in STZ-induced diabetic rats by treating with the combination of Glimepiride and NE. This may be due to alteration in the metabolism of Glimepiride either by enhancing absorption or by inhibiting CYP enzymes responsible for Glimepiride metabolism. No change in T_{max} of Glimepiride was found in NE treated group indicating that there is no alteration in rate of absorption of Glimepiride and the serum affinity of Glimepiride for albumin is 99.5% bound.

The mean serum glucose level and percentage glucose reduction of pretreated diabetic rats is shown in Table 2. The data revealed that there is a maximum reduction of serum glucose level in combination of NE with Glimepiride pretreated groups (52.8%), when compared to standard (glimepiride, 50.63%), and (NE 41.72%) alone pretreated groups at 6th hr, respectively. The increase in hypoglycemic action of concomitant administration of Glimepiride with NE was more in diabetic rats compared to alone treated drugs and with control group, which suggested that the enhancement of glucose reduction capacity of glimepiride in diabetic rats along with neem leaf extract.

Administration of glucose load (2 g/kg, p.o) increased serum glucose levels significantly (p<0.01) after 30 min of glucose loading in diabetic rats in OGTT. Glimepiride and NE treatment alone or in combination produced significant (p<0.01) increase in glucose threshold within 30 min of glucose loading and the effects were persisted till 120 min (Table 3).

The serum total antioxidant status of different pretreated groups in diabetic rats is shown in Figures 1. The combination of NE with Glimepiride group found gradually increased (p<0.01) total antioxidant status when compared with NE, Glimepiride alone pretreated groups and with control group at all time intervals of the study.

CONCLUSION:

The results of increased Glimepiride levels when given in combination with neem leaf extract may be due to decreased metabolism of Glimepiride. A significant long-term exposure of neem in diabetic animals being treated with glimepiride controlling diabeties in rats. Hence the combination has a beneficial effect in diabetic animals.

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***Corresponding Author:**
M. Vidyavathi*
Email: vidyasur@rediffmail.com