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Andrographolide Pretreatment Enhances the Bioavailability and Hypoglycemic Action of Glimepiride and Metformin

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Abstract

Herbal antidiabetic preparations are often used as an add-on therapy in diabetes. Hence, in the present investigation the effect of andrographolide (AD) on the pharmacokinetics and pharmacodynamics of glimepiride and metformin in normal as well as in STZ - induced diabetic rats was studied. In normal and diabetic rats the combination of glimepiride and metformin with AD increased significantly ($p < 0.01$) all the pharmacokinetic parameters, such as C_{max}, $AUC_{0 \text{ to } n}$, AUC_{total}, $t_{½}$, MRT and decreased the clearance, Vd markedly as compared with the control group. In pharmacodynamic studies, the combination of glimepiride and metformin with AD provided significant protection against the diabetes induced alterations in the biochemical parameters. In addition, the combination of glimepiride and metformin with AD also improved the total antioxidant status and decreased lipid peroxide levels significantly in diabetic rats compared with AD, glimepiride and metformin alone treated groups. The results revealed that combination of glimepiride and metformin with AD led to the enhancement of the bioavailability of glimepiride and metformin by inhibiting the CYP450 enzymes. In conclusion, add-on preparations containing AD may increase the bioavailability of glimepiride and metformin, which suggested that AD might be beneficial as an adjuvant to glimepiride and metformin in a proper dose, in diabetic patients and hence the doses should be monitored.

Keywords: Glimepiride; Metformin; Andrographolide; Pharmacokinetics; Pharmacodynamics

Introduction

The use of herbal medicine as alternative and/or complementary therapy in the western world is on the rise and gaining increasing popularity. As people often take different herbs in combination with prescribed modern medication, there is a potential for both pharmacokinetic and pharmacodynamic interaction [1]. Glimepiride and metformin both are used as oral hypoglycemic agents, widely used for the treatment of type 2 diabetes mellitus. The hypoglycemic effect of glimepiride and metformin were changed during co-administration with *Carica papaya* extract [2], and the bioavailability, hypoglycemic action of glimepiride was increased with piperine [3] and curcumin [4], thus there is a need to study the interaction between glimepiride, metformin and other drugs to avoid adverse effects.

Andrographolide (AD) is a major diterpenoid constituent of the plant *Andrographis paniculata*, known as "kalmegh", is widely used in Asia to treat the common cold, diarrhea, and fever associated with infectious diseases [5]. Many studies have shown *A. paniculata* and its major component, AD have various pharmacological activities, such as anti-inflammatory [6], anticancer [7], anti-platelet aggregation [8], anti-viral [9], antidiabetic [10] and hepatoprotective effects [11]. There are several *in-vitro* reports of AD on inhibition of CYP450s especially CYP3A4 [12], CYP2D6 [13], CYP3A1/2 [14], CYP1A2 [15] and CYP2C9 [16]. Hence, there is the possibility of AD for the metabolic inhibition of glimepiride and metformin, which are also completely metabolized by CYP2C9 [17] and CYP3A [18] microsomal liver enzymes.

In view of the effect of AD on CYP enzymes and also antidiabetic properties, its presence in herbal antidiabetic preparations may influence the pharmacokinetics and pharmacodynamics of glimepiride and metformin. Therefore, the aim of the present investigation was to study the effect of AD on pharmacokinetics and pharmacodynamics of glimepiride and metformin.

Experimental

Drugs and chemicals

Glimepiride, metformin and gliclazide were obtained as gift samples 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. Andrographolide (AD) was purchased from Yucca Enterprises, Mumbai. Ascorbic acid,

-diphenyl-β-picrylhydrazyl (DPPH), 1,1,3,3 – tetraethoxy propane (TEP), thiobarbituric acid and streptozotocin (STZ) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. Merck analytical kits were used to estimate the serum biochemical parameters. 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 rats of Wistar strain weighing 180 - 250g were purchased from Mahaveera enterprises, Hyderabad, India and used for the studies after obtaining the permission from institutional animal ethical committee (CPCSEA Reg. No.146/1999). 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 ± 5^0C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum.*

Pharmacokinetic study of glimepiride

Grouping of normal and diabetic rats and their pretreatment

Normal and diabetic rats were divided into 2 groups (n = 6). Group I was administered with glimepiride (1 mg/kg; b.w., p.o.) suspended in normal saline on 8^{th} day and group II was pretreated with AD (4.5 mg/kg; b.w., p.o.) for 7 days and on 8^{th} day with glimepiride (1 mg/kg) followed by AD.

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 [19] using heparinised capillary tubes at 0.5, 1, 2, 4, 6, 8, 12 and 24 h. Serum was separated after centrifugation at 8000 rpm for 15 min and stored at -20^0C until analysis.

Pharmacokinetic study of metformin

Grouping of normal and diabetic rats and their pretreatment

Normal and diabetic rats were divided into 2 groups (n = 6). Group I was administered with metformin (100 mg/kg; b.w., p.o.) suspended in normal saline on 8^{th} day and group II was pretreated with AD (4.5 mg/kg; b.w., p.o.) for 7 days and on 8^{th} day with metformin (100 mg/kg) followed by AD. Blood samples were collected and centrifuged same as mentioned in PK study of glimepiride.

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 [20]. 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.

HPLC analysis of glimepiride in normal and diabetic pretreated rats

Serum glimepiride concentration was determined by reverse phase HPLC [21]. The solvent delivery system was a shimadzu pump model LC-10AT (Shimadzu, Japan) and the analytical column used was Lichrosphere 100 RP C_{18} (125 4.0 mm i.d, 5 μ particle size). 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: 10 mM potassium dihydrogen ortho phosphate (pH 3.0 adjusted with ortho phosphoric acid) (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 quantitation.

HPLC analysis of metformin in normal and diabetic pretreated rats

Serum metformin concentration was determined by reverse phase HPLC [22]. Shimadzu pump model LC-10AT system with Lichrosphere 100 RP C_{18} was used with mobile phase consisting of 0.15M ammonium acetate: acetonitrile (90:10 v/v), at a flow rate of 1.0 mL/min and effluent was monitored at 236 nm. The total run time was less than 10 min.

Pharmacodynamic studies

Effect of AD with glimepiride on serum glucose in STZ-induced diabetic rats

STZ-induced diabetic rats were fasted overnight and divided into 4 groups (n = 6). The animals of group I (diabetic control, normal saline), group II (glimepiride, 1 mg/kg), group III (AD, 4.5 mg/kg), and group IV [AD (4.5 mg/kg) + glimepiride (1 mg/kg)] were treated orally with the material mentioned in the parenthesis of the respective group. The effect of the AD, glimepiride alone and their combinations on fasting blood glucose level was studied up to 24 h. Blood samples were drawn from the retro-orbital plexus of the rats at '0' (Initial fasting blood sample), 2, 4, 6, 8, 12 and 24 h after the treatment. The samples were analyzed for blood glucose using glucose oxidase-peroxidase method [23].

Effect of AD with metformin on serum glucose in STZ-induced diabetic rats

STZ-induced diabetic rats were fasted overnight and divided into 4 groups (n = 6). The animals of group I (diabetic control, normal saline), group II (metformin, 100 mg/kg), group III (AD, 4.5 mg/kg), and group IV [AD (4.5 mg/kg) + metformin (100 mg/kg)] were treated orally with the material mentioned in the parenthesis of the respective group. The effect of the AD, metformin alone and their combinations on fasting blood glucose level was studied up to 24 h. Blood samples were collected and analyzed as mentioned above.

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 in PD studies of glimepiride and metformin. The rats of all the groups were loaded with D - glucose (2.5 g/kg, p.o) 30 min after the treatment [24]. Blood samples were collected from the rats at 30, 60, 90 and 120 min after glucose loading for determination of blood glucose levels.

Assessment of different biochemical parameters in STZ-induced diabetic rats

Overnight fasted STZ-induced diabetic rats were divided into 4 groups (I - IV) same as mentioned in PD studies of glimepiride and metformin, they were treated once a day for 28 days (sub acute study) and their body weight, fasting blood glucose level, serum insulin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum cholesterol, serum triglyceride and serum total proteins were estimated [25]. During the study period, the body weight of the animals and blood glucose levels were recorded after 7, 14, 21 and 28 days of the treatment. Serum insulin, SGOT, SGPT, serum cholesterol, serum triglyceride and serum total protein levels were estimated after 28 days of the treatment.

Estimation of total antioxidant status in diabetic pretreated rats

The serum samples of sub acute study were used to determine the total antioxidant status by using DPPH method [26] for both PD studies of glimepiride and metformin. Ascorbic acid was used as a reference standard. The standard graph was prepared using different concentrations of ascorbic acid in water (y = 0.0018 x + 0.0116, r = 0.9953) and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Estimation of lipid peroxide levels in diabetic pretreated rats

The serum samples of sub acute study were used to determine the lipid peroxides by using Thiobarbituric acid reaction method [27] for both PD studies of glimepiride and metformin. The standard graph for determination of malondialdehyde levels was prepared using 1,1,3,3- tetraethoxy propane (TEP) reagent as the standard (y = 0.075 x - 0.0368, r = 0.9989) and the MDA content in the serum was expressed in nM/ml.

Statistical analysis

The Pharmacokinetic parameters were calculated by using Kinetica TM software (version 4.4.1, Thermo Electron Corporation, USA). 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 test using Graph Pad Prism 5 computer software. Values corresponding to p 0.05 were considered as significant.

Results

Pharmacokinetics of glimepiride in normal and diabetic pretreated rats

Table 1 summarizes the pharmacokinetic parameters of glimepiride in different groups of normal and diabetic rats. In normal and diabetic pretreated rats, compared with the control group (given glimepiride alone), the co administration of AD significantly ($p < 0.01$) increased C_{max} (1.95 times, 2.94 times), $AUC_{0\ to\ n}$ (2.95 times, 2.89 times), AUC_{total} (3.24 times, 2.95 times), $t_{1/2}$ (2.02 times, 1.27 times), MRT (1.52 times, 1.17 times), whereas the clearance (0.31 times, 0.34 times) and volume of distribution (0.63 times, 0.50 times) of glimepiride was decreased. The Tmax was not altered significantly in both normal and diabetic pretreated rats.

Table 1: Mean pharmacokinetic parameters of glimepiride in different groups of normal and STZ-induced diabetic rats.

PK parameter	Glimepiride		Glimepiride + AD	
	Normal	Diabetic	Normal	Diabetic
Cmax(μg/ml)	7.4±0.1	11.3±0.3	14.5±0.4**	33.3±1.2**
Tmax (h)	2	2	2	2
AUC_{0-n}(μg/ml h)	24.3±0.6	51.1±2.1	71.8±0.8**	147.5±3.6**
AUC_{tot} (μg/ml h)	24.6±0.7	51.8±2.0	79.8±1.3**	153.1±3.5**
$t_{½}$ (h)	1.6±0.2	1.7±0.3	3.3±0.2**	2.2±0.1**
MRT (h)	3.8±0.1	3.9±0.1	5.8±0.2**	4.6±0.1**
CL (ml/min)	38.7±1.4	18.6±1.1	12.3±0.1**	6.4±0.3**
Vd (ml)	90.4±7.5	44.8±4.5	57.4±2.5**	22.5±1.7**

fand volume of distribution (0.57 times, 0.31 times) of metformin was decreased.
The Tmax was not altered significantly in both normal and diabetic pretreated rats.

Table 2: Mean pharmacokinetic parameters of metformin in different groups of normal and STZ-induced diabetic rats.

PK parameter	Metformin		Metformin + AD	
	Normal	Diabetic	Normal	Diabetic
Cmax(μg/ml)	7.56±0.14	11.58±0.13	12.5±0.22**	36.65±0.14**
Tmax (h)	2	2	2	2
AUC_{0-n}(μg/ml h)	34.06±0.18	64.16±0.61	72.36±0.7**	328.15±0.75**
AUC_{tot} (μg/ml h)	36.07±0.27	71.52±0.74	80.83±1.37**	511.84±2.8**
$t_{½}$ (h)	2.6±0.11	3.34±0.04	3.27±0.13**	7.29±0.1**
MRT (h)	4.93±0.11	5.84±0.04	5.99±0.13**	11.67±0.12**
CL (L/h)	8.66±2.5	4.41±0.6	3.91±0.3**	2.58±0.1**
Vd (L)	32.09±8.1	21.14±3.8	18.31±4.5**	6.58±2.6**

All values are expressed as mean ± SD (n = 6).
*p < 0.05; **p < 0.01 considered as significant when compared with metformin control.
[a] Definitions of the parameters:
Cmax: Peak serum concentration; **Tmax:** 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; **Vd:** Volume of distribution.

Effect of AD and its combination on the hypoglycemic action of glimepiride and metformin

The mean serum glucose level and percentage glucose reduction of antihyperglycemic study of glimepiride and metformin pretreated diabetic rats is shown in Table 3 and Table 4. The glimepiride data reveals that there is a maximum reduction of serum glucose level in combination of AD with glimepiride pretreated group (51.2%), when compared to standard (glimepiride, 50.07%) and AD (38.08%) alone pretreated groups at 6th hr, respectively. However, combination of AD with glimepiride (1 mg/kg) showed sharp decrease ($p < 0.01$) in serum glucose levels at all the time points (Table 3).

Table 3: Comparison of mean serum glucose levels and percentage reduction of serum glucose level of Group II, Group III and Group IV with Group I in STZ - induced diabetic rats.

Group No	Treatment	Dose (mg/kg)	Blood glucose level (mg/dl) at different hours						
			0h	2h	4h	6h	8h	12h	24h
I	Control	---	334.5±1.6	332.8±1.6 (0.49%)	332.3±1.4 (0.66%)	331.2±1.2 (0.98%)	332.7±1.1 (0.54%)	333.2±1.2 (0.39%)	334.3±1.8 (0.07%)
II	Glimepiride	1mg/kg	342.5± 2.4	281.6± 3.9 (17.79%)**	226.3±4.6 (33.93%)**	171.2±4.8 (50.07%)**	255.1±11.3 (25.52%)**	291.5±4.9 (14.88%)**	331.1± 1.3 (3.35%)*
III	AD	4.5mg/kg	326.4±2.8	300.6±5.1 (7.9%)**	252.3±4.7 (19.64%)**	202.1±6.2 (38.08%)**	254.3±1.7 (22.09%)**	285.4±3.6 (12.56%)**	313.2±7.4 (4.04%)*
IV	AD + Glimepiride	4.5mg/kg +1mg/kg	355.2±5.1	293.6±4.5 (17.34%)**	242.3±5.2 (31.78%)**	173.4±3.8 (51.2%)**	215.5±2.5 (39.33%)**	238.2±7.2 (32.94%)**	281.2±6.4 (20.83%)**

Values are expressed as mean ± SD (n=6)

*p < 0.05; **p < 0.01 considered as significant when compared with Group I at respective time interval.

The metformin data reveals that there is a maximum reduction of serum glucose level in combination of AD with metformin pretreated group (41.72%), when compared to standard (metformin, 40.98%) and AD (31.45%) alone pretreated groups at 6th hr, respectively. However, combination of AD with metformin (100 mg/kg) showed sharp decrease (p < 0.01) in serum glucose levels at all the time points (Table 4).

Table 4: Comparison of mean serum glucose levels and percentage reduction of serum glucose level of Group II, Group III and Group IV with Group I in STZ - induced diabetic rats.

Group No	Treatment	Dose (mg/kg)	Blood glucose level (mg/dl) at different hours						
			0h	2h	4h	6h	8h	12h	24h
I	Control	---	270.5±8.7	269.9±9.2 (0.22%)	269.6±5.8 (0.33%)	268.9±7.2 (0.59%)	269.5±8.6 (0.37%)	269.4±6.9 (0.41%)	270.2±9.8 (0.11%)
II	Metformin	100mg/kg	282.1±9.2	247.8±7.8 (12.16%)**	205.2±5.4 (27.26%)**	166.5±8.1 (40.98%)**	192.8±6.5 (31.66%)**	239.8±9.5 (14.99%)**	276.3±8.4 (2.06%)*
III	AD	4.5mg/kg	260.4±7.4	241.6±9.6 (7.22%)**	210.3±5.7 (19.24%)**	178.5±8.1 (31.45%)**	195.2±10.2 (25.04%)**	232.4±9.1 (10.75%)**	257.2±6.2 (1.23%)*
IV	AD + Metformin	4.5mg/kg +100mg/kg	300.6±9.4	254.5±7.9 (15.34%)**	212.8±12.5 (29.21%)**	175.2±4.6 (41.72%)**	202.4±9.1 (32.67%)**	239.5±7.5 (20.33%)**	264.6±8.4 (11.98%)**

Values are expressed as mean ± SD (n = 6)

*p < 0.05; **p < 0.01 considered as significant when compared with Group I at respective time interval.

Increased glucose threshold in OGTT by treatments

In OGTT, administration of glucose load (2.5 g/kg, p.o) increased serum glucose levels significantly (p < 0.01) after 30 min of glucose loading in diabetic rats. Glimepiride, metformin and AD treatment alone or in combination produced significant (p < 0.01) increase in glucose threshold within 30 min of glucose loading and the effects persisted till 120 min (Table 5 and Table 6).

Table 5: Comparison of oral glucose tolerance and percentage reduction of serum glucose level of Group II, Group III and Group IV with Group I in STZ - induced diabetic rats.

Group No	Treatment	Dose (mg/kg)	Blood glucose levels (mg/dl) at different time intervals				
			0min	30min	60min	90min	120min
I	Control	---	424.43±2.57	475.7±7.2 (12.08%)	397.35 ±4.67 (6.3%)	382.60±2.47 (9.86%)	367.9±2.26 (13.32%)
II	Glimepiride	1mg/kg	403.67±2.49	464.5±8.5 (15.06%)**	333.22±2.0 (17.45%)**	278.70±2.67 (30.96%)**	241.63±2.13 (40.14%)**
III	AD	4.5mg/kg	396.2±4.5	474.6±5.6 (19.79%)**	342.4±5.5 (13.57%)**	306.2±3.7 (22.7%)**	281.3±1.4 (29.0%)**
IV	AD + Glimepiride	4.5mg/kg +1mg/kg	404.5±2.4	458.3±4.4 (13.30%)**	335.1±4.7 (17.16%)**	291.4±2.9 (27.96%)**	238.2±5.4 (41.11%)**

All values are expressed as mean ± SD (n = 6)

*p < 0.05; **p < 0.01 considered as significant when compared with Group I at respective time interval.

Table 6: Comparison of oral glucose tolerance and percentage reduction of serum glucose level of Group II, Group III and Group IV with Group I in STZ - induced diabetic rats.

Group No	Treatment	Dose (mg/kg)	Blood glucose levels (mg/dl) at different time intervals				
			0min	30min	60min	90min	120min
I	Control	---	305.2±5.8	361.6±9.4 (18.48%)	281.8 ±7.6 (7.67%)	275.1±9.2 (9.86%)	266.4±6.3 (12.71%)
II	Metformin	100mg/kg	332.6±8.2	384.2±7.5 (15.51%)**	272.5±8.8 (18.07%)**	235.4±6.5 (29.22%)**	206.8±5.8 (37.82%)**
III	AD	4.5mg/kg	302.8±9.7	395.8±8.1 (16.21%)**	266.2±7.5 (12.09%)**	238.4±10.6 (21.27%)**	210.6±7.8 (30.45%)**
IV	AD + Metformin	4.5mg/kg +100mg/kg	320.9±6.5	389.4±4.6 (21.35%)**	251.5±9.8 (21.63%)**	222.8±7.8 (30.57%)**	195.2±11.2 (39.17%)**

All values are expressed as mean ± SD (n = 6 *p < 0.05; **p < 0.01 considered as significant when compared with Group I at respective time interval.

Effect on different biochemical parameters in STZ-induced diabetic rats

There was a gradual diminution in body weight of animals in diabetic control group. The AD (4.5 mg/kg) alone treatment and its combination with glimepiride and metformin, standard drug treated groups showed a gradual and significant ($p < 0.01$) increase in the body weight from 7 days onwards. The increase in the body weight was observed till the end of the study (28 days) (Figure 1 and Figure 2).

Glimepiride (1 mg/kg), metformin (100 mg/kg), AD (4.5 mg/kg) and their combinations showed significant ($p < 0.01$) antihyperglycemic effect after 7th, 14th, 21st and 28th day of treatments. The effects of combinations were more pronounced than single drug treatment (Figure 3 and Figure 4).

There is a significant effect ($p < 0.01$) on the combination of AD with glimepiride in reducing serum GOT and GPT levels was maximum after 28 days showing 40.03% and 48.58% respectively and was well comparable to that of the standard drug, glimepiride (25.2%, 30.8%). Whereas in AD alone pretreated group also found maximum diminution in serum GOT and GPT levels after 28 days showing 32.71% and 36.54%. Also there is a significant effect ($p < 0.01$) on the combination of AD with metformin in reducing serum GOT and GPT levels was maximum after 28 days showing 34.86% and 38.35% respectively and was well comparable to that of the standard drug, metformin (26.41%, 31.25%).

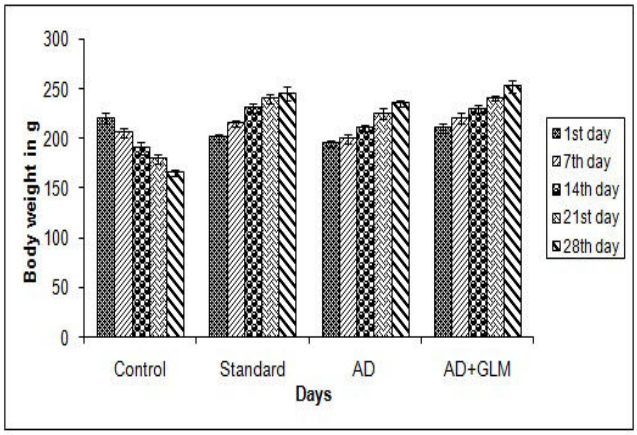

Figure 1: Effect of sub acute pretreatment of AD and glimepiride on body weights of STZ - induced diabetic rats

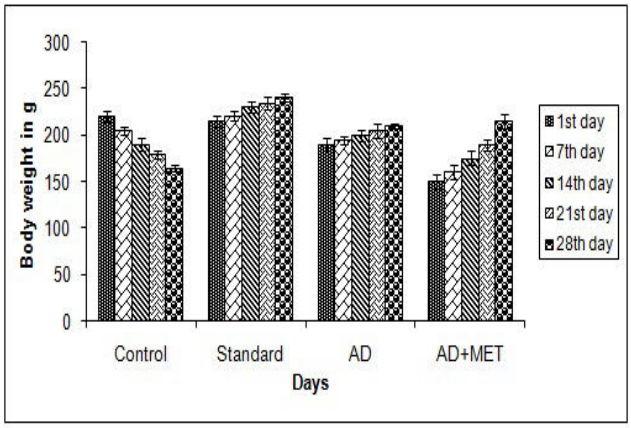

Figure 2: Effect of sub acute pretreatment of AD and metformin on body weights of STZ - induced diabetic rats

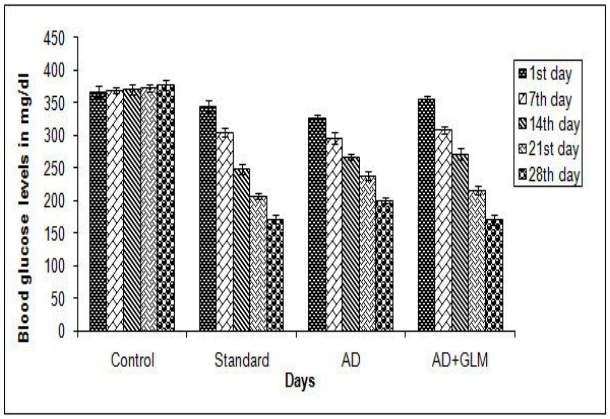

Figure 3: Effect of sub acute pretreatment of AD and glimepiride on blood glucose levels of STZ - induced diabetic rats

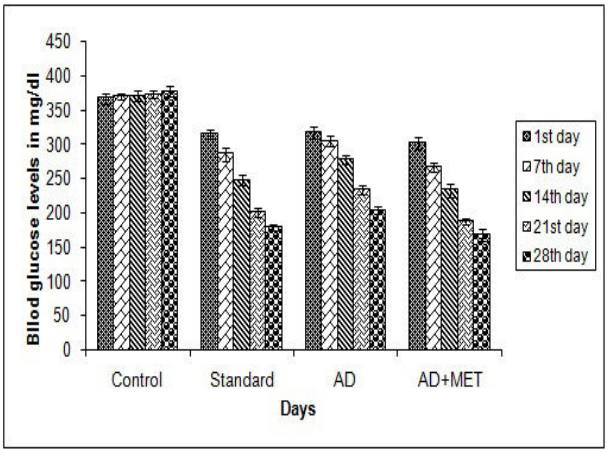

Figure 4: Effect of sub acute pretreatment of AD and metformin on blood glucose levels of STZ - induced diabetic rats

The percent reduction in serum triglyceride levels of AD in combination with glimepiride treated group was 44.75 while it was 41.71 in standard group (glimepiride) and 36.96 (AD, 4.5 mg/kg) alone pretreated group. Also the percent reduction in serum triglyceride levels of AD in combination with metformin treated group was 42.77 while it was 33.12 in standard group (metformin) and 39.46 (AD, 4.5 mg/kg) in alone pretreated group. However, the percentage reduction in combination of AD with glimepiride and metformin after 28 days was greater than that of the standard drugs and alone treated groups.

The percent reduction in serum cholesterol levels of AD in combination with glimepiride treated group was 48.82%, whereas it was 32.55% in standard group (glimepiride) and AD (35.84%) alone treated groups. Same as the percent reduction in serum cholesterol levels of AD in combination with metformin treated group was 41.73%, whereas it was 34.44% in standard group (metformin) and AD (37.76%) alone treated groups.

The significant increasing effect ($p < 0.01$) of the combination of AD with glimepiride group on serum total protein levels was maximum after 28 days showing 54.76% was well comparable to that of the standard glimepiride (51.1%) and AD (34.21%) alone pretreated groups. Also there is a significant increasing effect ($p < 0.01$) of the combination of AD with metformin group on serum total protein levels was maximum after 28 days showing 41.67% was well comparable to that of the standard metformin (36.36%) and AD (30.26%) alone pretreated groups.

The significant increasing effect ($p < 0.01$) of the combination of AD with glimepiride and AD with metformin groups on serum insulin levels was maximum after 28 days showing 51.69% and 43.53%, was well comparable to that of the standard glimepiride

(45.26%) and standard metformin (38.95%) and AD (35.16%) alone pretreated groups (Table 7 and Table 8).

Table 7: Comparison of different groups of AD and glimepiride on serum biochemical parameters in STZ - induced diabetic rats (Sub acute study).

Groups	Serum Insulin levels in IU/ml		SGOT (U/L)		SGPT (U/L)		Serum Triglyceride levels (mg/dl)		Serum Cholesterol (mg/dl)		Serum Total protein (mg/dl)	
	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days
Diabetic control	9.8±0.7	9.6±0.6 (2.04%)	132.5±8.2	141.4±4.2 (6.7%)	156.1±4.7	162.5±2.8 (4.1%)	153.6±3.0	157.2±5.6 (2.3%)	142.7±7.3	147.3±8.2 (3.22%)	4.0±0.2	3.8±0.7 (5.5%)
GLM	9.5±0.2	13.8±0.8** (45.26%)	128.6±6.2	96.2±7.1** (25.2%)	145.6±7.1	100.7±4.1** (30.8%)	158.2±5.7	92.2±4.3** (41.71%)	146.2±2.9	98.6±4.2** (32.55%)	4.5±0.6	6.8±0.5** (51.1%)
AD	9.1±0.4	12.3±0.5** (35.16%)	123.5±1.7	83.1±5.5** (32.71%)	144.5±3.8	91.7±1.9** (36.54%)	151.5±4.8	95.5±2.4** (36.96%)	153.2±2.9	98.3±2.7** (35.84%)	3.8±0.9	5.1±0.3** (34.21%)
AD + GLM	8.9±0.5	13.5±0.2** (51.69%)	120.4±5.1	72.2±2.7** (40.03%)	140.4±5.3	72.2±2.6** (48.58%)	155.1±3.8	85.7±4.3** (44.75%)	148.7±7.1	76.1±5.1** (48.82%)	4.2±0.4	6.5±0.5** (54.76%)

All values are expressed as mean ± SD, n = 6; Values given in the parenthesis are the percent increase or decrease in respective parameter level;
* p < 0.05; **p < 0.01 when compared with control at the respective time interval.

Table 8: Comparison of different groups of AD and metformin on serum biochemical parameters in STZ – induced diabetic rats (Sub acute study).

Groups	Serum Insulin levels in IU/ml		SGOT (U/L)		SGPT (U/L)		Serum Triglyceride levels (mg/dl)		Serum Cholesterol (mg/dl)		Serum Total protein (mg/dl)	
	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days	1 st day	After 28 days
Diabetic control	9.8±0.7	9.6±0.6 (2.04%)	132.5±8.2	141.4±4.2 (6.7%)	156.1±4.7	162.5±2.8 (4.1%)	153.6±3.0	157.2±5.6 (2.3%)	142.7±7.3	147.3±8.2 (3.22%)	4.0±0.2	3.8±0.7 (5.5%)
MET	9.5±0.8	13.2±1.5** (38.95%)	120.4±5.8	88.6±7.8** (26.41%)	140.8±7.4	96.8±5.9** (31.25%)	155.8±5.4	104.2±8.6** (33.12%)	150.4±5.8	98.6±9.6** (34.44%)	4.4±0.5	6.0±0.8** (36.36%)
AD	9.1±0.5	12.0±0.4** (31.87%)	127.5±5.8	89.4±6.2** (29.88%)	145.2±8.1	98.2±8.7** (32.37%)	150.8±6.1	91.3±5.8** (39.46%)	158.1±8.2	98.4±8.6** (37.76%)	3.8±0.5	4.95±0.7** (30.26%)
AD + MET	8.5±0.2	12.2±0.3** (43.53%)	130.8±6.8	85.2±5.4** (34.86%)	135.6±9.1	83.6±4.9** (38.35%)	142.4±7.2	81.5±4.6** (42.77%)	155.3±8.5	90.5±5.6** (41.73%)	3.0±0.2	4.25±0.4** (41.67%)

All values are expressed as mean ± SD, n = 6; Values given in the parenthesis are the percent increase or decrease in respective parameter level;
* p < 0.05; **p < 0.01 when compared with control at the respective time interval.

Total antioxidant status and lipid peroxide levels of different groups in diabetic rats

The serum total antioxidant status and lipid peroxide levels of different pretreated glimepiride groups in diabetic rats is shown in Figure 5 and Figure 6 and for metformin is shown in Figure 7 and Figure 8. The combination of AD with glimepiride and metformin groups found gradually increased ($p < 0.01$) in total antioxidant status when compared with AD, glimepiride and metformin alone pretreated groups and with control group at all time intervals of the study. The lipid peroxide levels were found decreased significantly ($p < 0.01$) in co administration of AD with glimepiride and metformin groups when compared with AD, glimepiride and metformin alone pretreated groups and with control group at all time intervals of the study.

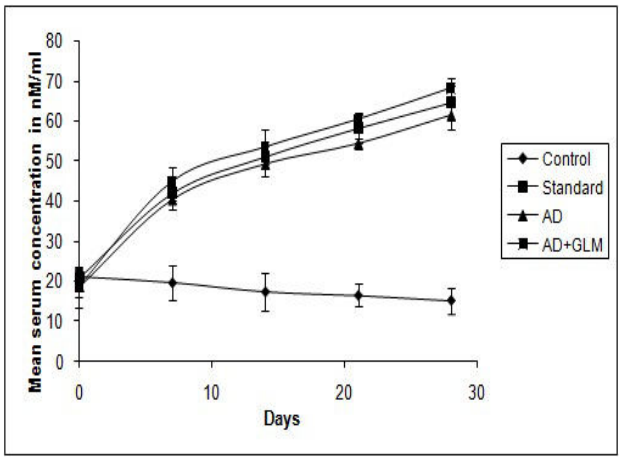

Figure 5: Effect of sub acute pretreatment of AD and glimepiride on total antioxidant status of STZ-induced diabetic rats

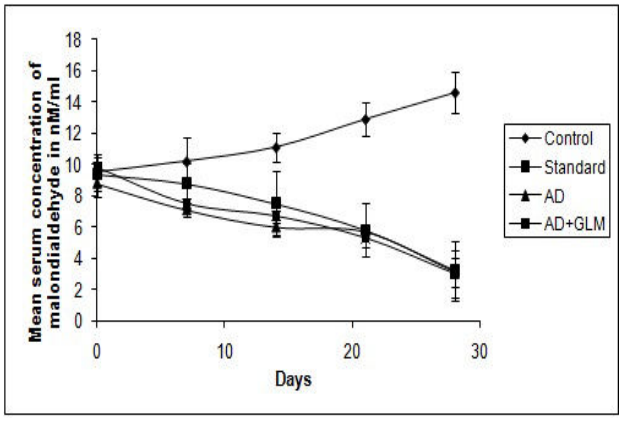

Figure 6: Effect of sub acute pretreatment of AD and glimepiride on lipid peroxide levels of STZ-induced diabetic rats

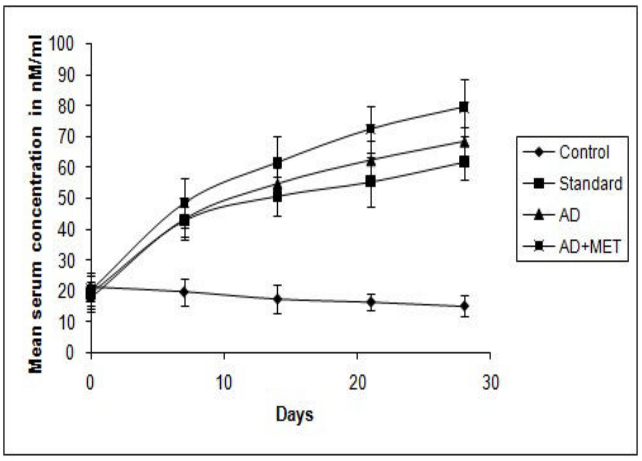

Figure 7: Effect of sub acute pretreatment of AD and metformin on total antioxidant status of STZ-induced diabetic rats

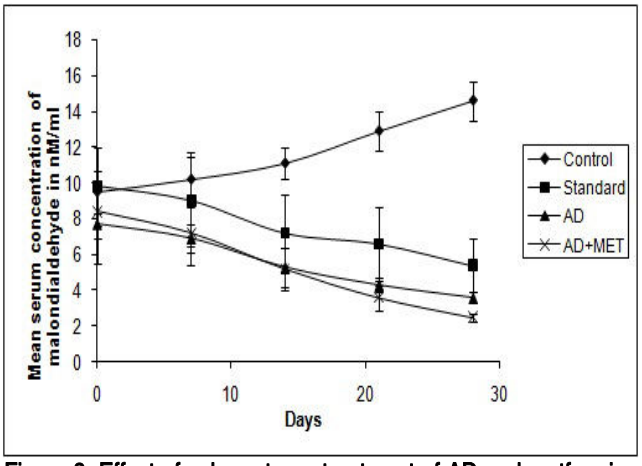

Figure 8: Effect of sub acute pretreatment of AD and metformin on lipid peroxide levels of STZ-induced diabetic rats

Discussion

In normal and STZ-induced diabetic rats, combination of glimepiride and metformin with AD led to a significant increase in pharmacokinetic parameters such as C_{max}, $AUC_{0 \text{ to } n}$, AUC_{total}, $t_{1/2}$ and MRT. This may be due to alteration in the metabolism of glimepiride and metformin either by enhancing absorption or by inhibiting CYP2C9 and CYP3A responsible for glimepiride and metformin metabolism. There is no change in T_{max} of glimepiride and metformin in both normal and diabetic rats indicating that there is no alteration in rate of absorption of glimepiride and metformin. This indicates that the decreased volume of distribution may not be due to displacement of glimepiride and metformin by AD. As there is no plasma protein binding interactions between AD, glimepiride and metformin, the decreased volume of distribution may be due to metabolic inhibition of glimepiride and metformin by AD. The present

investigations are in accordance with the earlier *in-vitro* studies of AD metabolic inhibition on CYP2C9 [16] and CYP3A [18] enzymes in human and rat liver microsomes.

The increase in hypoglycemic action by concomitant administration of glimepiride and metformin with AD was more in diabetic rats than when the drugs were used singly and with the control group, which suggests the enhancement of glucose reduction capacity of glimepiride and metformin in diabetic rats along with AD. In sub acute study, concomitant administration of AD with glimepiride and metformin produced more beneficial changes on body weight as well as on different serum biochemical parameters in STZ-induced diabetic rats. A significant improvement in body weight indicates the ability of combination of drugs and individual drugs to prevent loss of body weight in diabetic rats. It reveals that these drugs do not have any effect on degradation of depot fat to maintain the body weight [28]. Combination of glimepiride and metformin with AD and alone pretreated groups reduced significantly ($p < 0.01$) and gradually the blood glucose levels after 7 days to till the end of the study (28 days). This phenomenon clearly indicates that these drugs in combination control the hyperglycemic state of type 2 diabetes more effectively than alone treated drugs. The significant ($p < 0.01$) reduction in SGOT and SGPT levels further strengthens the antidiabetogenic effect of these drugs because increased gluconeogenesis and ketogenesis occur in diabetes, which may be due to high levels of SGOT and SGPT [29]. Concomitant administration of AD with glimepiride and metformin exhibited more antihypertriglyceridemic and antihypercholesterolemic activity than the drugs used individually. Further, the increased serum total protein level brought out by these drugs explains the antidiabetogenic effect, as a reduction in protein level takes place in diabetes due to deficiency of insulin, which stimulates uptake of aminoacids into muscle and increases protein synthesis [30]. The significant ($p < 0.01$) increase in serum insulin levels indicates that AD might have exhibited an antihyperglycemic effect like glimepiride and metformin, i.e. by insulin secretogogue activity [31].

The serum total antioxidant status and lipid peroxide levels suggests that the pretreatment of diabetic rats in combination or alone leads to significant increase in free radical scavenging capacity when compared to control group. This suggests that AD offer significant protection against the oxidative stress induced by diabetes and interfere with PK and PD.

Conclusion

The present study indicated that AD affects the metabolism of glimepiride and metformin, possibly by the inhibition of CYP2C9 and CYP3A. Combination of glimepiride and metformin with AD considerably enhances the glucose - lowering effect of glimepiride and metformin. Hence, glimepiride and metformin doses may require special attention if used along with AD and *Andrographis paniculata* extract containing herbal preparations to avoid complications.

Author's Contribution

Ciddi Veeresham was responsible to make a research concept, design of the study and corresponding author of the manuscript. Sujatha was responsible to data collection, acquisition of data, analysis of data and statistical of data.

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