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ANALYTICAL METHODS USED FOR THE ESTIMATION OF BLOOD GLUCOSE BY GOD/POD METHOD USING UV SPECTROSCOPY AT 520NM IN RATS AND RABBITS, BLOOD GLICLAZIDE ESTIMATION BY HPLC IN RABBIT SERUM.

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Abstract:

Glucose in blood is the most frequent analyzed parameter in a clinical chemistry laboratory. In Armed Forces Laboratories, copper reduction method (Modified Folin Wu) is commonly used. Here we have compared this method as well as O-Toluidine and GOD-POD method with reference UV-Hexokinase method. Both Modified Folin Wu and O-Toluidine showed upward deviation with substantial imprecision (CV=4.9% – 3.5% and 6% – 5.8% respectively) and inaccuracy (Average deviation = 5.76% and 10.68% respectively).

GOD-POD method was found linear (up to 500 mg/dl), with good precision (CV=0.7% to 1.4%) and accuracy (Average deviation= −0.97%). This method is simple, rapid, economical and sensitive, and can be adapted to a routine colorimeter.

KEYWORDS: Blood glucose, Diabetes mellitus, Glucose oxidase etc.

Introduction:

Drug interaction can be defined as "It may arise either from alteration of Pharmacological response or effect due to Pharmacodynamic or Pharmacokinetic of one drug by the other or from combination of their actions or effects"[1].

In modern life today, numerous studies have demonstrated that many patients receive multiple drug therapy with recognized potential .As the number of drugs in patient's therapeutic regimen increases, the greater is the risk of occurrence of drug interaction[2]. It is known that the incidence of adverse drug reaction to drugs rise from 4.2% when five or fewer drugs are used to 45% when twenty or more drugs are used .This may lead to enhanced or diminished effect of concomitantly used drugs may be useful or harmful .The useful drug interaction is illustrated by synergistic combination of drugs such a antibiotics or antihypertensive .Harmful drug interactions are ,unfortunately ,more numerous[3] .These drug interactions may result in severe adverse drug reaction, exaggerated pharmacological responses, toxic effects or reduced efficacy of drugs.

The characterization of drug-drug interactions has been a standard part of drug development programs for the last two decades. Before release to the general public, a new drug entity must be tested for its ability to modulate the pharmacokinetic or Pharmacodynamic effects of co-prescribed medications, and for the reverse effects of the see established medications on the new drug entity. Many clinically important drug-drug interactions involved.

METHODOLOGY:

ANALYTICAL METHODS USED IN THE STUDY

The analytical methods used for the estimation of blood glucose by GOD/POD method using UV spectroscopy at 520nm in rats and rabbits, blood gliclazide estimation by HPLC in rabbit serum.

Collection of blood samples from rats:

Materials:

1) Micro centrifuge tubes (1.5 ml capacity)

2) Micro capillary tubes (1 mm diameter).

3) Absorbent cotton

Blood was collected from the retro orbital plexus of rats. It is the best method, if small amounts (0.1 to 0.5 ml) of blood samples are required. A fine capillary is inserted gently in the inner angle of the eye, and then the capillary was slided under the eye ball at 45 degree angle and over the bony socket to rupture the fragile venous capillaries of the ophthalmic venous plexus. The passage is about 10 mm. The tip of the capillary is slightly retracted and the blood collected in the orbital cavity flows out from the capillary which is collected in a micro centrifuge tube. After collecting the desired volume, capillary is removed with simultaneous release of pressure by forefinger and thumb. Any residual blood droplet around the eye ball is wiped off by absorbent cotton swab. In this study un-anaesthetized animals were used because, anesthesia causes hyperglycemia by various mechanisms. Ether increases blood glucose levels by glycogenolysis in liver [55]. Halothane increases blood glucose by inhibiting release of insulin from pancreas, inhibit the effect of insulin on tissues and decreased rate of

glycogen synthesis in liver [56]

The same procedure was carried out for collection of blood samples from diabetic rats after induction of diabetic state by alloxan monohydrate.

3.2. Estimation of blood glucose in rats and rabbits

In this study the enzymatic; glucose oxidase-perixodase (GOD – POD) method [57] was used.

A. Glucose oxidase-peroxidase (GOD/POD) method

Glucose kit based on Trinder's method in which glucose oxidase (GOD) and peroxidase (POD) enzymes were used along with the chromogen 4-aminoantipyrine and phenol. This method is one step, simple and rapid.

Principle:

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction the oxygen liberated is accepted by the chromogen system to give a red colored quinoneimine compound. The red colour quinoneimine dye so developed is measured using Ultra-violet spectroscopy at 520nm

$$Glucose + H_2O \xrightarrow{Glucose\ oxidase} Gluconic\ acid + H_2O_2$$

Working reagent preparation: The contents of 1 vial of reagent-1 were transferred quantitatively to a clean black colored plastic bottle provided in the kit. The bottle was reconstituted with 50 ml of glucose diluent (Reagent-2).

Storage of working reagent: The working reagent is stable for 12 months from the date of reconstitution when stored at 2-8^{0}C.

Specimen collection: The collected blood was made to stand without adding any anticoagulant. The clot that is formed is disturbed using a glass rod and was then centrifuged at 3000 rpm for 10 min. The serum is separated and used for the analysis.

Equipment:

Programme: The basic assay parameters are:

Collection of blood sample from rabbits:

Blood samples were collected from the marginal ear vein of the rabbits for estimation of blood glucose and blood gliclazide. For this, rabbits were kept in wooden holders with their heads protruding out. The left ear,

for convenience, was shaved and blood vessels were dilated either by warming the ears on a low voltage electric lamp or by rubbing with a cotton swab. The dilated blood vessel of left marginal ear vein was punctured with a sharp syringe (22-24guaze) in the direction of venous blood flow. The blood was collected in micro centrifuge tubes.

3.2.C. Estimation of gliclazide in serum of rabbit by HPLC

A simple, sensitive HPLC method was developed for estimating the serum gliclazide levels. For pharmacokinetic studies, a method that allows an accurate measurement of low concentrations of gliclazide in serum is needed. Various analytical methods using high performance liquid chromatography (HPLC) and colorimetric assay[67] have been developed for the determination of gliclazide in biological samples. Each HPLC technique has its own advantages and disadvantages.

The present method is simple, sensitive and accurate which enables the determination of gliclazide even at ng/ml level in serum.

Chromatography: Typical chromatograms corresponding to individual blank serum and gliclazide (1µg) and diltiazem (2µg) {internal standard (IS)} were shown in

4 fig. No endogenous interfering peaks were visible at the retention times of gliclazide or diltiazem confirming the specificity of the analytical method. Both the analyte and the internal standard were well separated with retention times of 12.10 and 5.90 minutes, respectively. System suitability parameters for the method were as follows. Number of theoretical plates for gliclazide and IS were 4726.75 and 6124.34 respectively; tailing factor was less than 1.5 for both gliclazide and IS.

5 **Quantification:** The ratio of peak area of gliclazide to that of IS was used for the quantification of gliclazide in serum samples. The calibration curves were linear in the concentration range 50–1000 ng/ml. The calibration regression equation was $y = mx+c$, where y represents the peak area ratio of gliclazide to IS, x represents the concentration of gliclazide, m is slope of the curve and c is the intercept. The equation of the calibration curve obtained was $y = 0.0112x$; ($r^2=0.9995$) and its calibration curve was represented.

6 **Materials:** Gliclazide pure sample was gifted by Microlabs, Bangalore, India and diltiazem, pure samples was gifted by Sun pharmaceuticals, Mumbai respectively. Acetonitrile (HPLC grade) was purchased from Qualigens chemicals, Mumbai, India. Orthophosphoric acid (AR grade) and methanol (HPLC grade) were purchased from SD fine chemicals, Mumbai, India and Loba chemie pvt. Ltd., Mumbai, India respectively. Triple distilled water used for HPLC was prepared in the laboratory.

Standard solutions: Primary stock solution of 1 mg/ml of gliclazide and diltiazem were prepared in methanol and stored at 4 ^{0}C. Appropriate dilutions of gliclazide were made in mobile phase to produce concentrations of 10, 1 µg/ml and 500, 200, 100, 50 ng/ml. These dilutions were used to spike serum in the preparation of calibration curves. The IS working stock solution (100ng/ml) was made from primary stock solution using mobile phase for dilution. Calibration samples were prepared by spiking 100 µl of individual blank serum with appropriate amount of drug on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control rabbit serum in bulk of appropriate concentrations (50, 100,200, 500 and 1000 ng/ml) and stored at -4 ^{0}C.

Preparation of phosphate buffer (15Mm);

1. Solution A: Dissolve 2.04g of potassium dihydrogen phosphate in water to produce 1000ml.

2. Solution B: Dissolve 0.21g of disodium hydrogen phosphate in water to produce 1000ml.

Mix 96.4ml of solution and 3.6ml of solution B –pH5.5

Instrumentation: A gradient High Pressure Liquid Chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/VIS Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimionadzu) and RP C-18 column (GEMINI,250 mm x 4.6 mm I.D.; particle size 5 µm; YMC Inc., USA) was used. The HPLC system was equipped with the software "Class-VP series version 6.12SP2 (Shimadzu)".

Chromatographic conditions:

The mobile phase consisted of Acetonitrile (HPLC grade; supplied by M/s. Qualigens, Mumbai, India) and Phosphate buffer . The mobile phase components were filtered before use through 0.45µm membrane filter and pumped in the ratio of 50:50

{Acetonitrile: Phosphate buffer (15mM)} from the respective solvent reservoirs. The pH of the mobile phase was adjusted to 3.5±0.1 with Orthophosphoric acid. The flow rate of the mobile phase was maintained at 0.8 ml/min which yield column back pressure of 110-120 kgf and the column temperature was maintained at 40^0C. The eluent wasmonitored at 230 nm wavelength; sensitivity was set at 0.005 AUFS.

Construction of standard graph:

Concentration of gliclazide (ng/ml)	**Ratio of Gliclazide/ DILTAZEM(IS)**			
	Set – I	**Set - II**	**Set - III**	**Average±SEM**
50	0.56	0.599	0.679	0.62±0.03
100	1.067	0.984	1.157	1.07±0.04
200	2.048	2.117	2.092	2.09±0.02
500	5.562	5.805	5.723	5.69±0.07
1000	10.181	11.975	11.274	11.14±0.52

Table 3.1 Standard Plot for Gliclazide HPLC

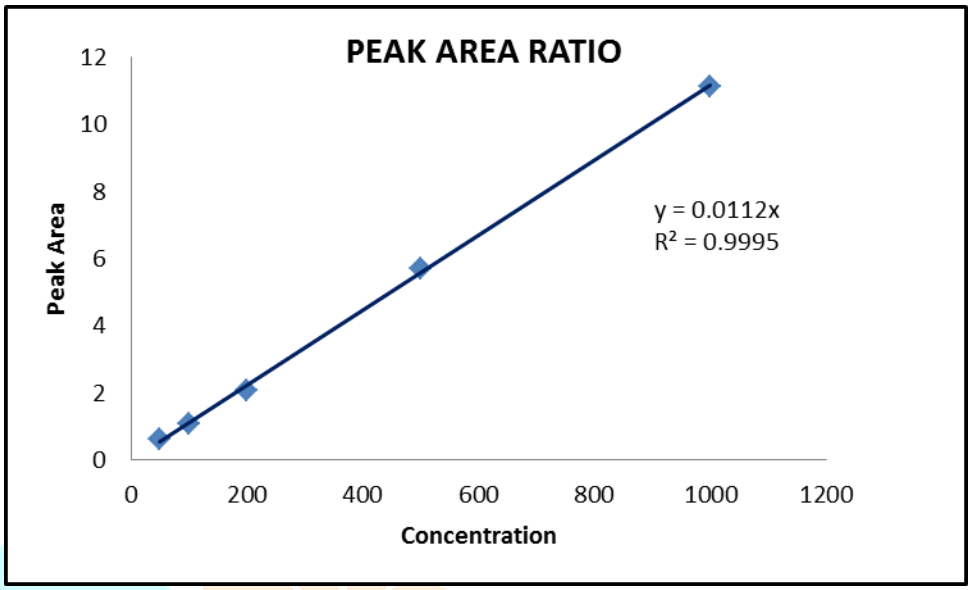
A linear relationship was found between the amounts of Gliclazide added to the serum samples and the corresponding ratios of AUC of Gliclazide to IS.

The graphical representation of the Gliclazide concentration vs. the corresponding AUC ratio value is shown in the following graph

For the routine estimation of blood Gliclazide concentration, serum sample (0.2 ml) was taken in 10ml stoppered test tubes and proceeded as described above. The concentration of Gliclazide in blood samples in the pharmacokinetic study can be directly read from the above standard graph or calculated from the linear relationship obtained as above. For the convenience, the blood Gliclazide concentrations were

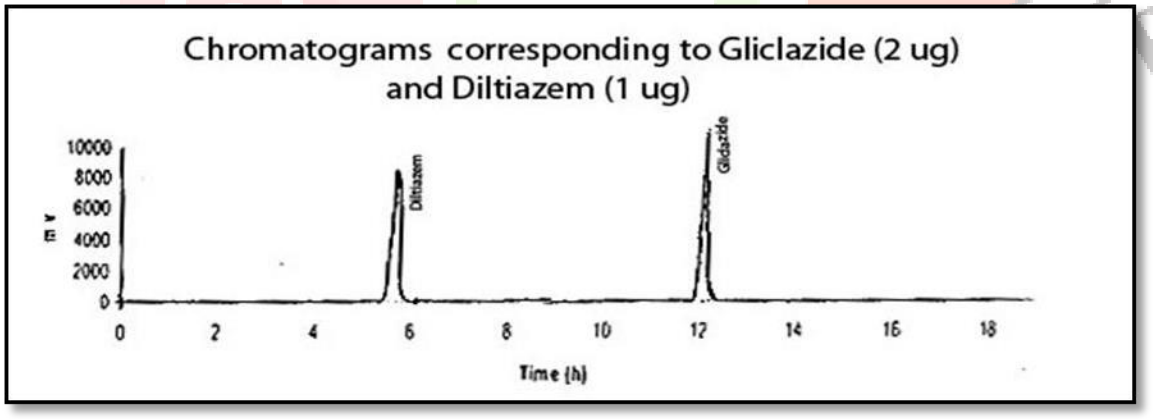
calculated from the linearrelationship.

Fig 3.1: Standard curve of Gliclazide



Where Y= ratio of AUC of gliclazide to IS, X= amount of gliclazide added to blood samples in ng. From this the gliclazide concentration per ml was calculated.

Figure 3.2: Represinting Chromatograms Corresponding to Gliclazide (2 µg) andDilteazem (1 µg)



Extraction from the serum:

To 100 µl of serum, 10 µl of diltiazem (internal standard, IS) working solution and 0.1 ml of Acetonitrile were added. Mixing for 10 sec, and the mixture was then centrifuged for 5 min at 5000 rpm. Supernatant liquid filter through 0.45 membrane filter using syringe filter and injected in to the HPLC column.. The eluent was detected by UV detector at 230 nm, and the data was acquired, stored and analyzed with the software

Class-VP series.

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Class-VP series.

DATA ANALYSIS

Blood gliclazide concentration vs. time data were analyzed by using the following equations. The parameters obtained thereby i.e. C_0 (blood concentration at time 0h), K_{el} (elimination rate constant) and K_a (absorption rate constant) were used in calculating the other pharmacokinetic parameters.

The elimination half life ($t_{½}$) was calculated using the relationship $t_{1/2} = 0.693/ K_{el}$. Volume of distribution was calculated using the relationship, V_d= F x Dose/C_0.

The area under the blood gliclazide vs. time curve , from 0 to 24 h was calculated by the trapezoidal rule and AUC 0-α was calculated using the same rule after extrapolation of the curve to infinity ($AUC = AUC_{0-24} + C_0 /Kel$) .

Total body clearance was determined by

$$CL = F\,X\frac{Dose}{AUC_{0-\alpha}}$$

The parameter T_{max} was calculated using the relationship.

$$T_{max} = \frac{2.303\,X\,Log\,(K_a/K_{el})}{K_a - K_{el}}$$

C_{max} was calculated using the formula

$$C_{max} = \left(F\,x\frac{Dose}{V_d}\right) X\, e - K_{el}\,X\,T_{max}$$

AUMC (The area under the first moment curve) was calculated by the trapezoidal rule and is a product of plasma gliclazide concentration x time (c t) V_S time curve from zero to infinity.

MRT (mean residence time) was calculated using the formula

$$MRT = \frac{AUMC}{AUC}$$

. **3.5. Statistical significance:**

The significance of the observed differences in pharmacokinetic parameters and percent blood glucose reduction of gliclazide between the drug treated and control rats (percent glucose reduction in both normal and diabetic rats) and rabbits (percent glucose reduction and pharmacokinetic parameters in normal rabbits) was assessed by student's paired t-test.

Value of $P < 0.05$ is considered for statistical significance to find out the difference between the parameters of comparison.

Gliclazide (5g) sample obtained from Microlabs, Bangalore, India and by Rosuvastatin (3g) sample obtained by were used. Blood glucose kits (Auto span) manufactured by Span diagnostics Ltd, Surat, India were purchased from a diagnostic kits and suppliers. Gliclazide solution in distilled water was prepared by dissolving 50 mg of gliclazide in a few drops of 0.1N sodium hydroxide then made up to 10 ml with distilled water.

Animals Used in the Study:

Inbred adult Wistar albino rats of either sex were procured from Mahaveer Enterprises, Hyderabad, India. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC). Standard animal pellet diet manufactured by Rayan's biotechnologies pvt Ltd, Hyderabad, India was used for feeding the animals

Procedure:

Albino rats of either sex weighing between 180-280 g were used in the study. They were divided into 3 groups each consisting of 6 rats. Rats were maintained on uniform diet and at room temperature with 12 h /12 h light and dark cycle. They were housed in polypropylene cages. Rats were fed with standard animal pellet diet and water *ad libitum.* The rats were fasted for 18 h prior to the experiment with water *ad libitum*. During the experiment water was also withdrawn.

Methods:

For the pharmacodynamic study, rats were divided into three groups of six each. They were fasted for 18 h before the experiment and both water and food were withdrawn during the experiment. Group I/II/III were administered with Gliclazide namely 3.6mg/200g bd.wt.(1/2TD), 7.2mg/200g bd.wt.(TD) and, 14.4mg/200g bd.wt.(2TD). The same groups were administered with Rosuvastatin weight respectively after a washout period of one week. Later group II was treated with the combination of Gliclazide ½ TD and Rosuvastatin 0.72mg/200g body weight with a washout period of one week. Diabetes was induced in rats by the administration of Alloxan monohydrate in two doses i.e., 100mg and 50mg/Kg bodyweight intraperitonially for two consecutive

days. Six Alloxan induced diabetic rats were treated with a combination of Gliclazide TD and Rosuvastatin with a washout period of one week between the treatments. The blood samples were collected in rats from retro orbital puncture at 0, 1, 2, 3, 4, 6, 8, 10, and 12h and were analyzed for blood glucose by GOD/POD method using commercial glucose kits. To find out the influence of selected dose of Rosuvastatin on pharmacodynamics and pharmacokinetics of Gliclazide, a group of 6 normal healthy rabbits were used. The rabbits were fasted for 18 h before the experiment and both water and food were withdrawn during the experiment. They were administered with 5.6mg/ 1.5 kg body weight of Gliclazide (1/2 TD), orally. The blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, 16 and 24 h time intervals. The same group after a wash out period of one week was administered with the combination of Rosuvastatin and Gliclazide orally; 30 minutes prior to Gliclazide administration (2.8mg /1.5kg body weight). The blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 12, 16 and 24 h time intervals. They were analyzed for blood glucose levels by GOD-

POD method using UV spectroscopy at 520 nm. and blood gliclazide levels were estimated by HPLC.

RESULTS

Study of Influence of Drugs on Normal Rats

a) With Gliclazide: The Gliclazide was used as prototype drug of sulphonylurea in the present study of herb-drug interactions .Gliclazide induced hypoglycaemia was studied by administering it in different doses namely 3.6mg/200g bd.wt.(1/2TD), 7.2mg/200g bd.wt.(TD) and, 14.4mg/200g bd.wt.(2TD) for the dose response effect in the actual laboratory conditions. Gliclazide ½ TD produced 35.21±0.74 % and 36.34±0.74% reduction in blood glucose at 2h and 8h respectively. Gliclazide TD produced 66.78±0.56% and 50.14±11.46 % reduction in blood glucose at 6h and 8h respectively. Gliclazide 2TD produced 23.17±5.7.% and .22.91±6.94% reduction in blood glucose at1h and 12h respectively.

Gliclazide has shown dose dependent effect on blood glucose of rats. After establishing the dose response effect, a dose of 3.6mg/200g bd.wt.(1/2 TD) was selected for studying the interaction in other sets of experiments. The blood glucose levels observed with ½ TD, TD and 2TD of Gliclazide were shown in table 5.1a,5.2a, 5.3a and respectively. The results of dose effect relationship were given in the tables 5.1b,5.2b, 5.3band the graphical representation was done in ***figure 5.1***. The dose of ½ TD of Gliclazide was selected based on ideal blood glucose reduction which was about 30%.

Table 5.1a Blood glucose levels (mg/dl) with GLICAZIDE (1/2 TD) in normal rats (N=6)

Time(h)	Blood glucose levels in rats (mg/dl)						Mean±SEM
	R_1 (180g)	R_2 (204g)	R_3 (196g)	R_4 (190g)	R_5 (210g)	R_6 (212g)	
0	77	80	76	80	90	99	83.21±3.35
1	53	55	58	56	65	70	59.5±2.45
2	50	52	49	50	59	65	54.17±2.39
3	54	56	54	54	61	72	58.5±2.66
4	52	53	55	55	64	72	58.5±2.93
6	53	55	54	53	62	65	57±1.92
8	50	51	50	49	58	61	53.16±1.87
10	60	63	66	67	73	75	67.33±2.14
12	69	70	72	70	85	88	75.66±3.16

Table 5.1b Percent Blood glucose reduction with GLICAZIDE (1/2 TD) in normal rats (N=6)

Time(h)	Percent blood glucose reduction in rats						Mean±SEM
	R_1 (180g)	R_2 (204g)	R_3 (196g)	R_4 (190g)	R_5 (210g)	R_6 (212g)	
0	-	-	-	-	-	-	-
1	31.16	31.25	23.6	30	27.7	29.2	28.81±1.17
2	35.06	35	35.05	37.5	34.4	34.3	35.21±0.47
3	29.8	30	28.9	32.5	32.2	27.2	30.1±0.81
4	32.46	33.7	27.6	31.2	28.8	30.3	30.67±0.92
6	31.16	31.25	28.9	33.7	31.1	34.3	31.74±0.80
8	35.05	36.25	34.2	38.7	35.5	38.3	36.34±0.74
10	22.07	21.2	13.1	16.2	18.8	24.2	19.26±1.67
12	10.3	12.5	5.2	12.5	6.2	11.1	9.6±1.29

Table 5.2a Blood glucose levels (mg/dl) with GLICAZIDE (TD) in normal rats (N=6)

Time(h)	Blood glucose reduction in rats						Mean±SEM
	R_1 (250g)	R_2 (200g)	R_3 (200g)	R_4 (210g)	R_5 (220g)	R_6 (220g)	
0	90	100	101	80	85	95	91.84± 3.42
1	95	95	59	76	54	61	73.34±7.4
2	80	66	85	69	70	75	74.17±2.9
3	70	59	47	70	63	50	59.84±3.99
4	53	48	55	42	60	53	51.83±2.5
6	50	22	19	35	26	26	29.67±4.6
8	81	17	17	50	48	53	44.34±9.93
10	75	28	34	74	84	23	53±11.21
12	83	33	25	65	79	30	52.5±10.69

Table 5.2b Percent Blood glucose reduction with GLICAZIDE TD in normal rats (N=6)

Time(h)	Percent blood glucose reduction in rats						Mean±SEM
	R_1 (250g)	R_2 (200g)	R_3 (200g)	R_4 (210g)	R_5 (220g)	R_6 (220g)	
0	-	-	-	--	-	-	-
1	6.2	4.9	40.7	5	35.4	34.8	21.17±7..12
2	11.7	34.3	15.5	13.7	17	20.7	18.81±3.34
3	22.4	41.3	53.3	12	25.5	46.6	33.58±6.45
4	41.7	51.8	45.6	47.2	29.4	43.6	43.21±3.12
6	44.8	77.6	81	56	69.4	71.8	66.78±5.6
8	10.8	83.2	82.5	37.2	43.7	43.4	50.14±11.46
10	17.3	72	66	6.7	11	75.5	41.41±13.43
12	7.9	67.1	75.1	18.7	7	68.3	40.68±13.33

Table 5.3a Blood glucose levels (mg/dl) with GLICAZIDE (2 TD) in normal rats (N=6)

Time(h)	Blood glucose levels in rats (mg/dl)						Mean±SEM
	R-1 (260g)	R-2 (220g)	R-3 (230g)	R-4 (250g)	R-5 (240g)	R-6 (220g)	
0	90	88	100	110	100	100	98±2.98
1	53.5	55	86	100	80	88	77.08±7.02
2	90	100	90	67	85	98	83.33±4.41
3	50	79	94	88	98	71	80±6.59
4	80	90	102	90	110	80	92±4.47
6	57	111	84	82	91	95	86.67±6.64
8	65	95	68	91	93	93	84.17±5.13
10	39	80	62	80	103	90	75.67±8.36
12	45	85	70	85	95	79	76.5±6.51

Table 5.3bPercent Blood glucose reduction with GLICAZIDE (2 TD) in normal rats (N=6)

Time(h)	Percent blood glucose reduction in rats						Mean±SEM
	R-1 (260g)	R-2 (220g)	R-3 (230g)	R-4 (250g)	R-5 (240g)	R-6 (220g)	
0	-	-	-	-	-	-	-
1	46.5	38.1	14.6	8.36	20	11.5	23.17±5.7
2	10	-13	10.6	38.5	14.8	7.9	11.47±6.13
3	49.3	10.3	6.3	19.8	2	28.8	19.41±6.53
4	19.8	-1.6	-2	17.9	-10.4	19.9	7.26±5.01
6	42.6	-26.4	16.6	25.1	8.5	5	21.07±8.63
8	35.2	-7.1	3.21	17.4	6.4	7.1	10.36±5.39
10	60.4	9.8	38.5	27	-3	9.8	23.75±8.62
12	54.5	4.17	30.4	22.7	4.8	20.9	22.91±6.94

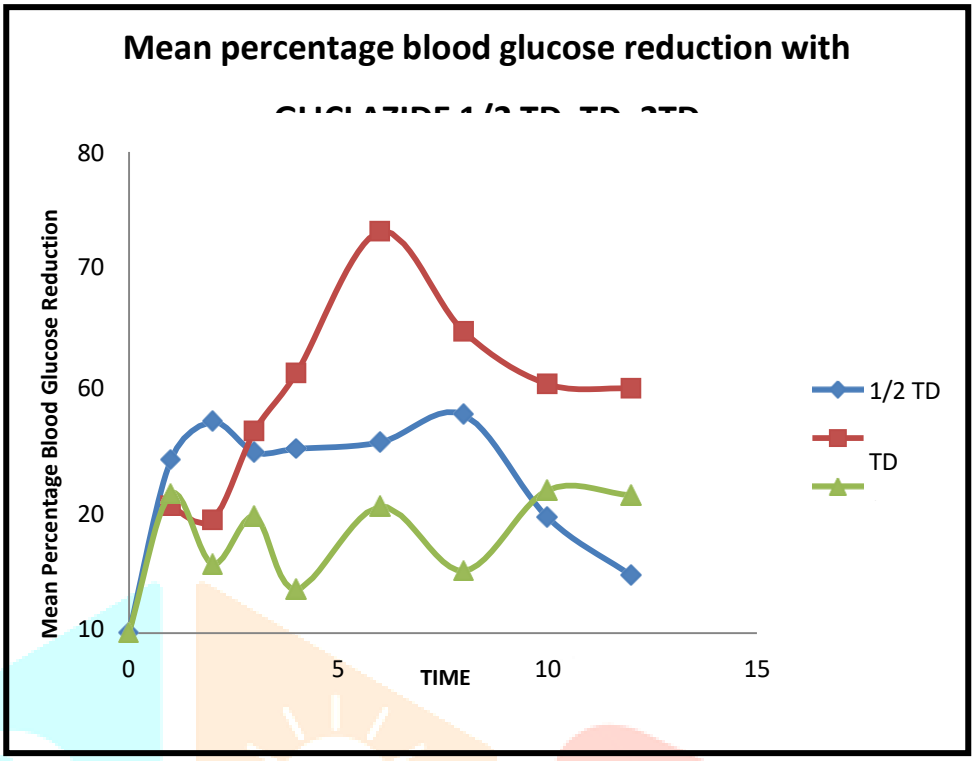

Fig: 5.1Mean percentage blood glucose reduction with GLICLAZIDE 1/2 TD, TD, 2TD

b) With *Rosuvastatin* The results of the blood glucose levels and the percent blood glucose reduction with½ TD 0.36mg/200g, TD 0.72mg/200g, 2TD 1.44mg/200g, of Rosuvastatin were tabulated in tables 5.4a-4b, 5.5a-5b, 5.6a-6b respectively. This treatment produced a minimum reduction of 0.54%, 3.01% and 6.58 %, and a maximum reduction of 4.52%, 13.54% and 26.63% respectively. Percent blood glucose reduction with different doses of Rosuvastatin in normal rats were presented graphically in ***figure 5.2***.The dose of 2mg/200g bodyweight of Rosuvastatin was selected based on ideal blood glucose reduction which is about 10%.

Table 5.4 a Blood glucose levels (mg/dl) with Rosuvastatin (1/2 TD) in normal rats (N=6)

Time(h)	Blood glucose levels in rats (mg/dl)						Mean±SEM
	R-1 (182g)	R-2 (204g)	R-3 (190g)	R-4 (195g)	R-5 (210g)	R-6 (220g)	
0	80	82	80	70	86	76	79 ± 2.23
1	79	80	78	67	85	76	77.5 ± 2.43
2	78	81	77	66	85	75	77±2.62
3	78	81	76	65	84	70	75.66±2.88
4	78	79	75	64	82	71	74.83±2.65
6	79	81	76	65	84	74	76.5±2.71
8	80	80	78	66	85	76	77.5±2.604
10	75	80	70	65	80	75	74.16±2.38
12	79	81	76	67	85	75	77.16±2.50

Table 5.4 b Percent Blood glucose reduction with Rosuvastatin (1/2 TD) in normal rats (N=6)

Time(h)	Percent blood glucose reduction in rats						Mean±SEM
	R-1 (182g)	R-2 (204g)	R-3 (190g)	R-4 (195g)	R-5 (210g)	R-6 (220g)	
0	-	-	-	-	-	-	-
1	1.25	0.22	0.25	1.47	1.16	0	0.73±0.25
2	1.25	1.22	1.28	2.94	1.17	1.32	1.53±0.28
3	2.5	1.22	1.28	2.94	1.17	1.82	1.82±0.30
4	2.5	3.66	3.85	5.88	4.65	6.58	4.52±0.31
6	1.25	1.22	2.56	4.41	2.32	2.63	2.39±0.47
8	0	2.44	0.25	2.94	1.16	0	1.29±0.52
10	1.25	2.44	1.58	4.41	2.44	1.32	1.18±0.48
12	0	1.22	2.56	1.47	1.16	1.32	0.54±0.22

Table 5.5 a Blood glucose levels (mg/dl) with Rosuvastatin (TD) in normal rats (N=6)

Time(h)	Blood glucose levels in rats (mg/dl)						Mean±SEM
	R_1 (195g)	R_2 (210g)	R_3 (200g)	R_4 (196g)	R_5 (185g)	R_6 (212g)	
0	70	74	72	78	72	68	72.33±1.54
1	68	72	70	76	70	65	70.17 ± 1.65
2	65	69	68	73	68	64	67.84±1.54
3	62	65	66	68	63	58	64.67±1.54
4	59	63	63	68	63	58	62.33±1.59
6	61	64	66	67	64	59	63.50±1.34
8	62	65	68	69	64	60	64.67±1.54
10	65	66	64	68	66	62	65.67±0.91
12	68	68	66	72	68	63	67.50±1.31

Table 5.5 b Percent Blood glucose reduction with Rosuvastatin TD in normal rats (N=6)

Time(h)	Percentage Blood glucose reduction in rats (mg/dl)						Mean±SEM
	R_1 (195g)	R_2 (210g)	R_3 (200g)	R_4 (196g)	R_5 (185g)	R_6 (212g)	
0	-	-	-	--	-	-	-
1	2.86	2.70	2.78	2.56	2.78	4.41	3.01±0.30
2	7.14	6.76	5.56	6.41	5.56	5.88	6.21±0.29
3	11.43	12.16	8.33	10.26	9.72	11.76	10.61±0.64
4	15.72	14.86	12.50	12.82	12.50	14.70	13.85±0.63
6	12.86	13.51	8.34	14.10	11.11	13.23	12.19±0.95
8	11.43	12.16	5.56	11.54	11.11	11.76	10.59±1.11
10	7.14	10.82	11.12	12.84	8.33	8.82	9.84±0.93
12	2.86	8.11	8.34	7.69	5.56	7.36	6.65±0.94

Table 5.6 a Blood glucose levels (mg/dl) with Rosuvastatin (2 TD) in normal rats (N=6)

Time(h)	Blood glucose levels in rats (mg/dl)						Mean±SEM
	R-1 (196g)	R-2 (190g)	R-3 (194g)	R-4 (210g)	R-5 (194g)	R-6 (200g)	
0	76	80	78	82	92	94	83.67±3.34
1	73	76	72	75	85	88	78.17±2.98
2	70	72	66	70	83	80	73.50±2.93
3	68	68	63	67	80	77	70.50±2.92
4	60	63	55	58	68	65	61.50±2.13
6	65	65	60	60	70	69	64.83±1.90
8	66	64	58	65	73	70	66.0±2.31
10	65	63	62	66	71	72	66.50±1.84
12	63	60	63	69	73	75	67.17±2.71

Table 5.6 b Percent Blood glucose reduction with Rosuvastatin (2 TD) in normal rats (N=6)

Time(h)	Percent blood glucose reduction in rats						Mean±SEM
	R-1 (196g)	R-2 (190g)	R-3 (194g)	R-4 (210g)	R-5 (194g)	R-6 (200g)	
0	-	-	-	-	-	-	-
1	4.28	5	7.69	8.53	7.61	6.38	6.58±0.74
2	8.57	10	15.38	14.63	9.78	14.89	12.21±1.37
3	11.43	15	19.23	18.29	13.04	18.08	15.84±1.42
4	22.86	21.25	29.48	29.26	26.08	30.85	26.63±1.74
6	15.72	18.75	23.07	26.82	23.91	26.59	22.48±1.97
8	14.28	20	25.64	20.73	20.65	25.53	21.14±1.87
10	15.72	21.25	20.51	19.51	22.82	23.40	20.54±1.23
12	18.57	25	19.23	15.85	20.65	20.21	19.92±1.34

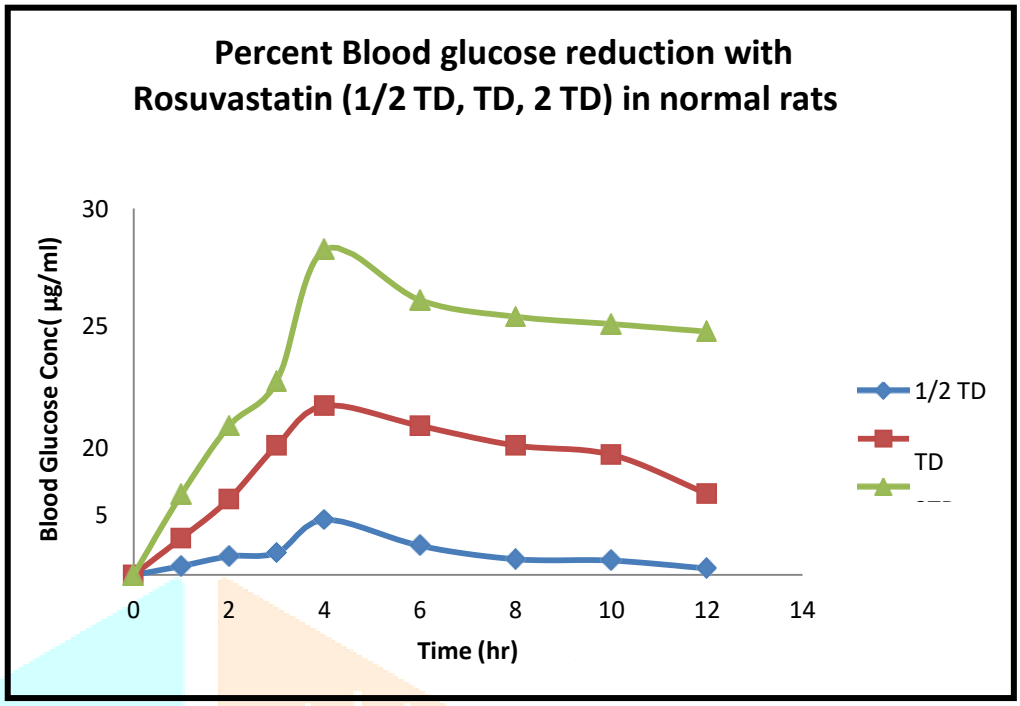

Fig: 5.2Mean percentage blood glucose reduction with ROSUVASTATIN1/2 TD, TD, 2TD

Drug interactions are usually seen in clinical practice and are generally due to under medication or over medication. Drug interactions may be pharmacokinetic or pharmacodynamic . We studied the influence of Rosuvastatin on the pharmacodynamics and pharmacokinetics of Gliclazide in animal models rats and in rabbits. The normal rat model served to quickly identify the interaction and diabetic rat model served to validate the same response in the actually used condition of the drug. The rabbit model is another dissimilar species to validate the occurrence of the interaction.

Gliclazide produced biphasic response in the rat model when administered alone, which may due to its biliary excretion and enterohepatic cycling[72] such effect was not seen in rabbit model. Gliclazide is known to produce hypoglycemic activity by pancreatic[73](stimulating insulin secretion by blocking K^+ channels in the pancreatic â cells) and extra pancreatic[74] (increasing tissue uptake of glucose) mechanisms.

Rosuvastatin produced a slight anti-hyperglycemic action when administered alone in normal rats and this may be due to its activity on the insulin secretion and it also enhanced hypoglycemic effects produced by Gliclazide when administered in combination. Since Rosuvastatin is known to be metabolized to a major extent by CYP 450 2C9 by which Gliclazide is also metabolized primarily, the interaction might be at the level of their metabolism. Rosuvastatin may compete with Gliclazide for metabolism by CYP 450 2C9 and

delay the metabolism of Gliclazide leading to its enhanced effect. The metabolites of Gliclazide namely hydroxy and carboxy Gliclazide are pharmacologically inactive. Hence inhibition of Gliclazide metabolism improves itsunchanged level and pharmacological action, which is seen in the present study.

Further Gliclazide is eliminated through renal (80%) and biliary (20%) routes. Rosuvastatin and its major metabolites are eliminated primarily through bile 92±10% being excreted in the feces and 4.9±1.6% [76]. Hence there was possibility for interaction between Rosuvastatin and Gliclazide at biliary excretion also. However the drug Rosuvastatin did not change the pattern of biphasic response of Gliclazide indicating that it did not interfere with the reabsorption of Gliclazide in its enterohepatic circulation in rats. In the presence of above drugs sustained hypoglycemic activity of Gliclazide was observed compared to Gliclazide control.

CONCLUSION

- Dose related hypoglycemic effect was observed for Gliclazide with ½ TD, TD and 2TD/200g dose in normal rats.
- Rosuvastatin produced dose dependent hypoglycemia at the doses 0.36mg, 0.72mg, 1.44mg/200g bodyweight in normal rats.
- Biphasic peak effect was observed with Gliclazide in rats which might be due to its reabsorption during its enterohepatic circulation in its excretion which is similarto human.
- Rosuvastatin produced the hypoglycemic effect, and when given in combination with Gliclazide, enhanced the hypoglycemic effect of Gliclazide in normal rats.
- The similar results were observed in Alloxan induced diabetic rats with Gliclazide before and after Rosuvastatin 0.72mg/200mg. Rosuvastatin produced anti- hyperglycemic effect with peak effect at 3 h. The results indicated the presence ofdiabetic condition alter the effect of above drugs on blood glucose.

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