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Pharmacokinetic Interactions Between Concomitantly Administered Metformin And Itopride In Rats

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Abstract

Gastroparesis is a syndrome characterized by delayed gastric emptying, in the absence of mechanical obstruction of the stomach. Diabetes mellitus (DM) is responsible for almost one third of cases of gastroparesis. Gastroparesis typically develops after at least 10 years of evolution of DM, and often is associated with other complications. The present study aimed to investigate the safety, reliability of Metformin and possible drug interaction with Itopride when they were administered as combination treatment. The study was conducted on healthy Wistar and streptozotocin induced diabetic rats. A simple and sensitive high performance liquid chromatographic method was developed for the simultaneous estimation of Metformin and Itopride in rat plasma and also to estimate possible pharmacokinetic parameters of these drugs after oral administration. There was no significant difference in the Metformin alone and combination with Itopride and Itopride alone and combination with Metformin on day 1 and day 8 respectively. There is no significant change in t_{max}, c_{max}, AUC $_{(0-t)}$ and AUC $_{(o\text{-}inf)}$, $t_{½}$, Cl/f and V/f on day 1 and day 8 respectively in both diabetic and healthy rats. From the above results it can be concluded that the concurrent administration of these two drugs have potential benefit in the treatment of Diabetes and Gastroparesis. In addition, due to their insignificant pharmacokinetic interaction the combinational therapy can be safe and highly advantageous in Gastro paresis patients with diabetes.

Keywords: Valsartan Metformin, Itopride, Gastroparesis, Diabetes mellitus, Pharmacokinetics..

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and an increased risk of complications from vascular diseases [1-3]. Diabetes occur either due to decreased synthesis of insulin (Type-1) or due to defective secretion of insulin from beta cells of islets of langerhans (Type-2) [4]. Literature study reveals that diabetic patients developing multiple pathology such as fungal infection, cardiovascular disorders, nephropathy, retinopathy, neuropathy, sexual impotence, hyperacidity, constipation and respiratory tract infections [5]. Gastroparesis is a syndrome characterized by delayed gastric emptying, in the absence of mechanical obstruction of the stomach. The cardinal symptoms include postprandial fullness, nausea, vomiting, and bloating. Diabetes mellitus (DM) is responsible for almost one third of cases of gastroparesis. Gastroparesis typically develops after at least 10 years of evolution of DM, and often is associated with other complications. It may cause severe symptoms and result in nutritional compromise, impaired glucose control and a poor quality of life.. Symptoms connected to gastroparesis are reported by 5 to 12% of DM.

Drug interactions have been reported to be the fourth to sixth leading cause of death in hospitalized patients in United States [6]. Drug–drug interactions may occur when more than one drug is administered in a patient to treat a single ailment or multiple ailments. The concomitant use of several drugs is often desired to obtain a therapeutic objective or to treat co-existing diseases. Simultaneous use of several drugs may lead to drug–drug interactions, the net result of interaction may be enhanced or diminished effects of one or both the drugs or the appearance of anew effect that is not seen with either drug alone [7]. There are several diseases those require lifelong treatment such as diabetes and hypertension. Patients with such diseases will often need to be administered drugs for treatment of other co-existing diseases, either for a short period or lifelong [8]. Metformin has an oral bioavailability of 50–60% under fasting conditions, and is absorbed slowly. Peak plasma concentrations (Cmax) are reached within one to three hours of taking immediate-release Metformin and four to eight hours with extended-release formulations. The plasma protein binding of Metformin is negligible, as reflected by its very high apparent volume of distribution (300–1000 l after a single dose). Steady state is usually reached in one or two days. Metformin is metabolized [9] and it is excreted unchanged in the urine and does not undergo hepatic metabolism

(no metabolites have been identified in humans) or biliary excretion9, 10. Itopride undergoes rapid and extensive absorption with levels of Itopride peaking in the blood plasma after only 35 minutes. Itopride is primarily eliminated via the kidneys having an elimination half-life of approximately 6 hours. In the present study, two diseases (diabetes and Gastroparesis) those may co-exist and require chronic treatment with the possibilities of occurrences of interactions between the concurrently used drugs.

Materials and methods

Materials

Drugs and Chemicals

Metformin and Itopride were procured from Matrix laboratories as a gift sample. Streptozotocin (STZ) was purchased from Sigma Chemical Co. The glucose estimation (GOD- POD) kit (excel diagnostic, Hyderabad) was procured from drug store. All HPLC grade solvents (methanol and water) were procured from finar chemicals Ltd., Ahmadabad. All chemicals used were analytical grade.

Animal study

Healthy Wistar rats were (Weighing 200-220 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee.

Induction of Experimental Diabetes

Male Wistar rats (200-250gms) were fasted for 16 hours before the induction of diabetes with Streptozotocin (STZ), a valuable agent for induction of Type-1 Diabetes mellitus. Animals (n=6) were injected intraperitoneal with 0.22 - 0.25ml of freshly prepared solution of STZ (60 mg / ml in 0.01 m citrate buffer, pH 4.5) at a final dose of 60mg / kg body wt. The diabetic state was assessed in STZ - treated rats by measuring the non-fasting serum glucose concentration after 48 hours. Only rats with serum glucose levels greater than 300 mg / dl were selected and used in this experiment.

Study Design

The rats were grouped as follows

Group I : Metformin alone in single dose / day in diabetic rats.
Group II : Itopride alone in single dose / day in diabetic rats.
Group III : Itopride alone in single dose / day in normal healthy rats
Group IV : Metformin and Itopride concomitant administration in diabetic rats as a single dose / day.

Collection of blood samples

After administration of the drugs, blood samples of 0.5 ml were drawn from each anesthetized (isoflurane) rat at pre-determined time intervals was collected from the retro-orbital plexus using a capillary tube into pre-labeled eppendorf tubes containing 10% of K_2EDTA anticoagulant (20 µL). The time intervals for the sample collection were 0 (Pre dose), 0.5, 1, 2, 4, 6, 8 and 24 hrs (post dose), Equal amount of saline was administered to replace blood volume at every blood withdrawal time.
Plasma was obtained by centrifuging blood samples by using cooling centrifuge (REMI ULTRA) at 3000 rpm for 5 minutes. The obtained plasma samples were transferred into pre-labeled micro centrifuge tubes and stored at 20 C until bio analysis of pharmacokinetic and pharmacodynamic parameters. As described above, all the procedures were followed on day 8 also. Pharmacokinetic parameters were calculated by non-compartmental analysis by using Win Nonlin® 5.1 software. Concentrations obtained from the above bio-analytical method were
Compiled [12,13].

Method of analysis

Preparation of plasma samples for HPLC analysis

Rat plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of Acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the Acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µ1 of mobile phase was injected for HPLC analysis.
For HPLC An Inertsil ODS 3V, 250x4.6 mm, C18 column with 5 µm particle size and the Mobile Phase consisting of phosphate buffer and Methanol Ratio 70:30 respectively. The flow rate was 0.8

ml/min and the effluents were monitored at 215 nm. Internal standard Phenformin was used. The retention time of internal standard (Phenformin) Metformin and Itopride in plasma were 9.8min, 7.2min and 3.3min respectively[14].

Standard calibration curve of Metformin and Itopride in rat plasma

Different concentration (0.05, 0.1, 0.5, 1, 5, 10, 20, 40 µg/ml) of Metformin, Itopride in plasma were prepared for calibration curve. The samples were treated as above for protein precipitation method and peak areas of Metformin and Itopride were noted down. The peak area ratios obtained at different concentrations of the Metformin and Itopride were plotted using UV – Vis detector at 215 nm.

Pharmacokinetic analysis

Plasma concentration versus time data was analyzed using standard non-compartment analysis.AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life [t½]. Volume of distribution (V/f) total clearance (Cl/f) and mean residence time for each subject using a non-compartmental analysis by using Win Nonlin® 5.1 software.

Statistical analysis

Statistical comparisons for the pharmacokinetic – pharmacodynamic study among, Metformin, Itopride, Itopride alone and in combination groups and plasma concentration – response study among concentrations and time were carried out with student's paired T-Test a value of $P<0.05$ was considered to be statistically significant. Data were reported as mean ± S.E.M linear regressions were used to determine the relationship between total plasma concentrations and pharmacokinetic and pharmacodynamic parameters. The mean concentration versus time profile of Metformin and Itopride in rat plasma is shown in Figures 1, 2, 3, 4, 5 & 6 14[12].

Results and Discussion

Table 1: Mean ± S.E.M, pharmacokinetic parameters of Metformin alone and in Combination with Itopride on day 1

parameters	Metformin alone	Metformin combination with Itopride	Level of significance(p<0.05)
C_{max} (µg)	25.74±0.39	26.03±0.12	NS
t_{max} (h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	272.45±0.74	276.37±0.52	NS
AUC_{o-inf} (µg /ml/h)	368.82±2.50	356.15±1.85	NS
T1/2 (h)	11.56±0.28	10.64±0.09	NS
CL/f(ml/h/kg)	2.196±0.015	2.23±0.01	NS
V/F(ml/kg)	40.63±0.26	37.56±0.25	NS

NS-Not significant

Table 2: Mean ±S.E.M, pharmacokinetic parameters of Metformin alone and in Combination with Itopride on day 8

parameters	Metformin alone	Metformin combination with Itopride	Level of significance(p<0.05)
C_{max} (µg)	31.92±0.22	32.41±0.1	NS
t_{max} (h)	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	337.36±0.38	343.38±0.49	NS
AUC_{o-inf} (µg /ml/h)	396.36±0.63	413.49±0.74	NS
T1/2 (h)	8.496±0.024	9.08±0.018	NS
CL/f(ml/h/kg)	1.961±0.009	1.92±0.05	NS
V/F(ml/kg)	26.67±1.27	27.89±0.32	NS

NS- Not significant

Table 3: Mean ± S.E.M, pharmacokinetic parameters of Itopride in diabetic versus healthy male Wistar rats on day 1

Parameters	Itopride in diabetic rats	Itopride in healthy rats	Level of significance(p<0.05)
$C_{max\ (\mu g)}$	3.92±0.03	2.72±0.03	NS
$t_{max\ (h)}$	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	33.49±0.20	21.9±0.118	NS
AUC_{o-inf} (µg /ml/h)	52.23±1.36	27.49±0.808	NS
T1/2 (h)	15.22±0.49	12.03±1.11	NS
CL/f(ml/h/kg)	15.475±0.401	26.71±0.18	NS
V/F(ml/kg)	359.16±2.38	497.65±3.09	NS

NS- Not significant

Table 4: Mean ±S.E.M, pharmacokinetic parameters of Itopride in diabetic versus healthy male Wistar rats on day 8

Parameters	Itopride in diabetic rats	Itopride in non diabetic rats	Level of significance(p<0.05)
$C_{max\ (\mu g)}$	4.80±0.04	3.65±0.06	NS
$t_{max\ (h)}$	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	44.11±0.225	38.22±0.09	NS
AUC_{o-inf} (µg /ml/h)	61.57±0.43	67.37±0.336	NS
T1/2 (h)	12.49±0.186	48.92±0.0908	NS
CL/f(ml/h/kg)	13.01±0.08	11.58±0.04	NS
V/F(ml/kg)	255.8±2.192	346.6±0.639	NS

NS- Not significant

Table 5: Mean ± S.E.M, pharmacokinetic parameters of Itopride alone and in Combination with Metformin in diabetic rats on day 1

Parameters	Itopride alone	Itopride with Metformin	Level of significance (p<0.05)
$C_{max\ (\mu g)}$	3.92±0.03	3.783±0.02	NS
$t_{max\ (h)}$	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	33.49±0.016	31.62±0.20	NS
AUC_{o-inf} (µg /ml/h)	52.33±1.37	42.16±0.52	NS
T1/2 (h)	15.22±0.49	11.57±0.27	NS
CL/f(ml/h/kg)	15.475±0.40	19.03±0.266	NS
V/F(ml/kg)	359.16±2.233	337.66±4.25	NS

NS- Not significant

Table 6: Mean ±S.E.M, pharmacokinetic parameters of Itopride alone and in Combination with Metformin in Diabetic rats on day 8

Parameters	Itopride alone	Itopride with Metformin	Level of significance (p<0.05)
$C_{max\ (\mu g)}$	4.80±0.04	4.615.±0.04	NS
$t_{max\ (h)}$	2±0	2±0	NS
AUC_{o-t} (µg/ml/h)	33.5±0.2253.2	41.6±0.25	NS
AUC_{o-inf} (µg /ml/h)	52.6±0.43	59.76±0.88	NS
T1/2 (h)	15.23±0.18	12.89±0.29	NS
CL/f(ml/h/kg)	15.43±0.08	13.48±0.03	NS
V/F(ml/kg)	358.65±2.16	272.1±2.26	NS

NS- Not significant

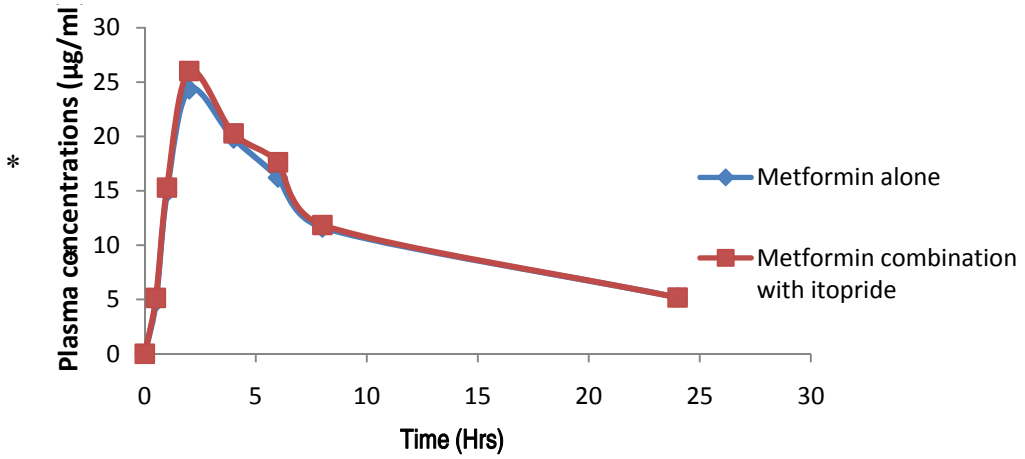

Figure 1: Mean Plasma concentrations (µg/ml) of Metformin alone and combination with Itopride on day 1 in Diabetic male Wistar rats (n=6)

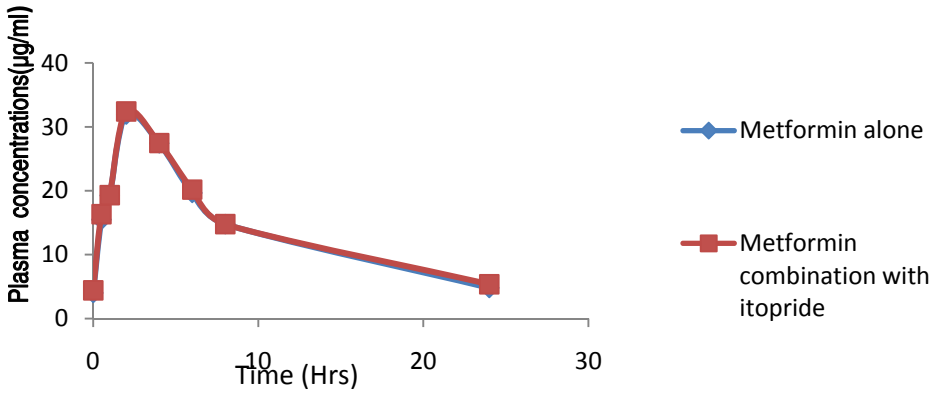

Figure 2: Mean Plasma concentrations (µg/ml) of Metformin alone and combination with Itopride on day 8 in Diabetic male Wistar rats (n=6)

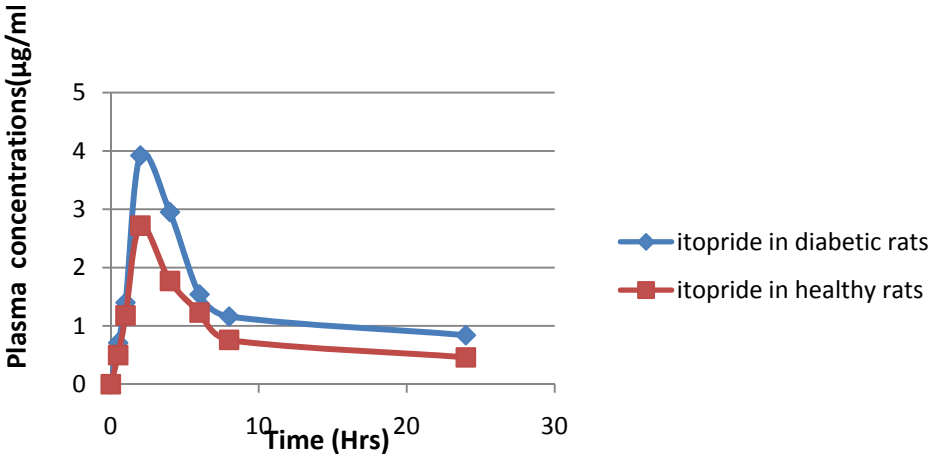

Figure 3: Mean plasma concentrations (µg/ml) of Itopride in healthy rats versus diabetic male Wistar rats on day 1(n=6)

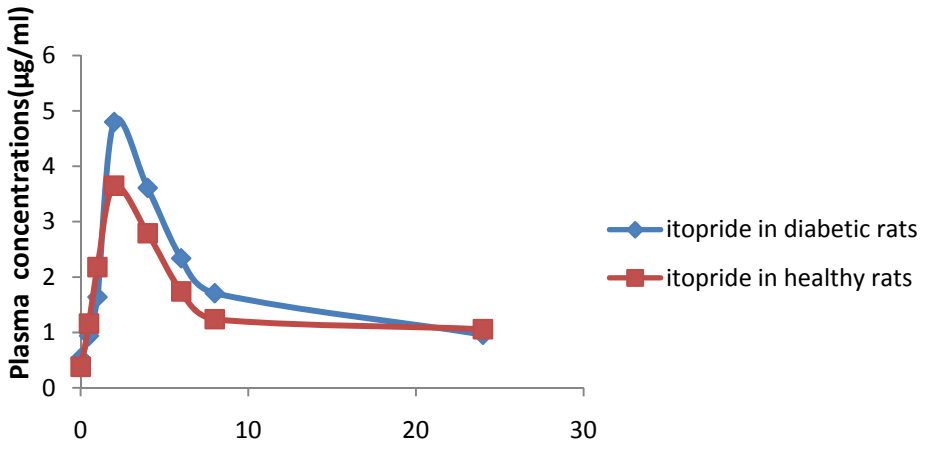

Figure 4: Mean plasma concentrations (µg/ml) of Itopride in healthy rats versus diabetic male Wistar rats on day 8(n=6)

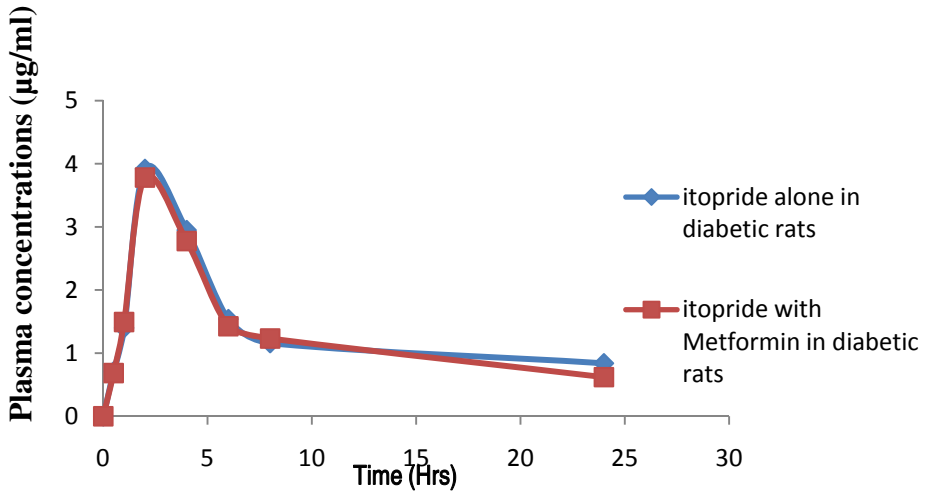

Figure 5: Mean plasma concentrations (µg/ml) of Itopride alone and combination with Metformin on day 1 in diabetic male Wistar rats (n=6)

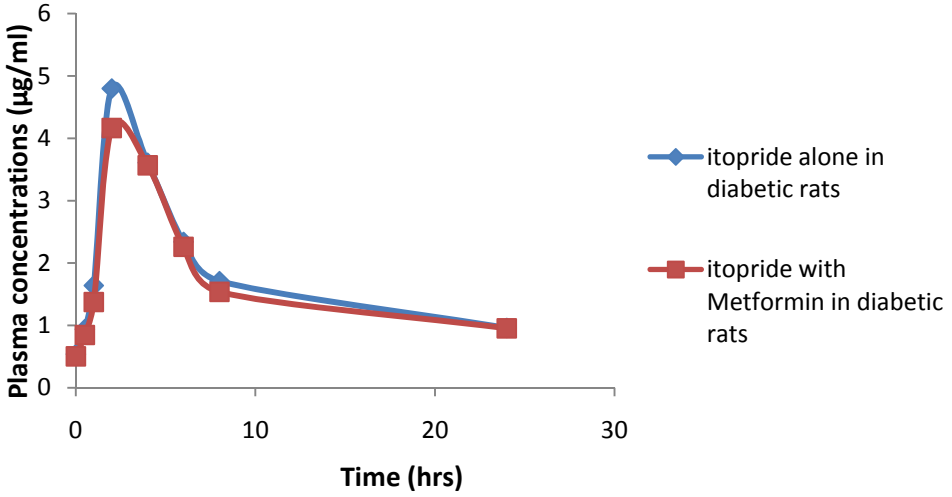

Figure 6: Mean plasma concentrations (µg/ml) of Itopride alone and combination with Metformin on day 8in diabetic male Wistar rats (n=6)

In the present study, Metformin is completely absorbed after oral administration with peak plasma concentration of 24.34±0.3μg/ml after 2hrs of dosing on day 1. In combination with Metformin and Itopride on day 1, the peak plasma concentration of Metformin 26.03±0.12μg/ml occurred 2 hr after dosing. There was no significant increase in peak plasma concentration levels. Similarly Itopride is completely absorbed after oral administration with peak plasma concentration 3.92±0.03μg/ml occurred 2hr after dosing on day 1 in combination with Metformin and Itopride on day 1. The peak plasma concentration of Itopride 3.783±0.02 μg/ml occurred 2hr after dosing. There was no significant increase in the peak plasma concentration levels similarly on day 8 of Metformin alone and with combination of Metformin with Itopride on day 8. Peak plasma concentration are 31.92±0.22 μg/ml and 32.41±0.10μg/ml respectively similarly Itopride on day 8 and combination with Metformin concentrations are 4.80 ± 0.04 μg/ml and 4.615 ± 0.04μg/ml respectively. There was no significant difference in peak plasma concentration on day 8 (P>0.05). The results were showed in from figure 1 to 6.

A significant differences was observed between diabetic and healthy Itopride treated rats on day 1 and day 8 respectively (P<0.05) on oral administration of Itopride alone and with combination of Metformin. With Itopride on day 1 showed a 2% increase in the AUC_{0-t} of Metformin compared to combinational treatment similarly. Itopride on day 1 and with combination Metformin with Itopride on day 1 administration resulted in an increase in the AUC_{0-24} of Itopride compared with combinational treatment. Similarly on day 8 of Metformin and Itopride in combination treatment were 1.65% and 2.8% increase in the AUC_{0-24} respectively.

The mean AUC_{0-t} of Itopride in diabetic (HL) rats was 33.49± 0.20 μg/ml /h and 44.11 ±0.22 μg/ml/h which was reduced to 21.9±0.11μg/ml/h and 38.22 ±0.09μg/ml/h Itopride in healthy rats on day 1 and day 8 treatment (P<0.05) respectively. There was slight decrease in the clearance (CL/F) rate of Metformin in combination compared with Metformin alone by 2.52% and 0.92% on day 1 and day 8 respectively. Similarly there was slight decrease in the clearance (CL/F) of Itopride in combination compared with Itopride alone by 4.92% on day 1. On day 8 a slight increase in the clearance (CL/F) of Itopride in combination compared with Itopride alone by 4.6%.

The half life was similar with alone and combination treatment on day 1 and day 8. All these changes were not statistically significant (P>0.05).

The mean clearance (C1/F) was 15.475±0.401(ml/h/kg) and 26.71±0.18(ml/h/kg) which was reduced to 13.01±0.08(m1/h/kg) and 11.58±0.042(m1/h/kg) upon treatment of Itopride in diabetic rats and healthy rats on day 1 and day 8 respectively.

Volume of distribution was increased 3.8% and 1.72% in Metformin alone compared with Metformin and Itopride on day 1 and day 8 respectively. similarly Itopride in diabetic rats and healthy rats resulted 359.16 and 497.65 on day 1 respectively Itopride alone on day 1 and combination with Metformin on day 1 administration resulted in 1.5% increases of volume distribution (ml/kg) in alone Itopride group treated rats similarly on day 8 administration resulted in 5.5 increases of volume of distribution in alone Itopride treated rats. The results were showed from table 1to 6.

Conclusion

The present study results suggest that the Concomitant administration of Metformin with Itopride has no significant pharmacokinetic interactions at absorption, distribution metabolism and excretion so it can be concluded that the concomitant administration of Metformin and Itopride is effective and safe for treatment, thus suggesting cautionary use in diabetic and Gastroparesis condition.

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