Research Article

Drug Interaction of Repaglinide and Mangiferin in Rats

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ABSTRACT

Herbal antidiabetic preparations are often used as an add-on therapy in diabetics. Mangiferin indica (Anacardiaceae) stem bark contains a rich content of Mangiferin and is used traditionally in Indian Ayurvedic system to treat diabetes. Mangiferin has been reported to be potent Alpha- Glucosidase inhibitor that also shows modulation of CYP450 enzyme that inhibits CYP3A4 enzyme. Repaglinide is a Meglitinides, class of drugs used treat diabetes type 2.They bind to an ATP-dependent K+ (KATP) channel on the cell membrane of pancreatic beta cells in a similar manner to sulfonylureas but at a separate binding site. It lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the beta cells. This depolarizes the beta cells, opening the cells' calcium channels, and the resulting calcium influx induces insulin secretion. and emerged as a novel oral Antidiabetic agent in recent past. It is metabolised by multiple Cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9. Hence it is speculated that Mangiferin may influence the kinetic, diabetic and Hepatotoxicity effect of Repaglinide which is particularly crucial, as any increment in its plasma level may raise safety concerns. Lipid profile of Repaglinide(0.2mg/kg) with Mangiferin(200mg/kg) were evaluated in Alloxan induced diabetic rats. The blood samples were collected from diabetic rats and Fasting blood sugar, TG, TC, LDL and HDL was estimated along with Hepatotoxicity study. Hepatotoxicity activity was estimated and Mangiferin significantly reduces the level of AST and ALT enzymes alone and in combination with Repaglinide. The study indicates that Therapeutic drug monitoring has to be required to readjust the therapeutic dose of Mangiferin and Repaglinide when they used concomitantly.

Keywords: Mangiferin, Repaglinide, Alloxan, Pharmacodynamic, Antidiabetic, Hepatotoxicity.

INTRODUCTION

Drug interaction is the modification of the effect of one drug (object drug) by the prior or concomitant administration of another drug (precipitant drug). Drug interaction may either enhance or diminish the intended effect of one or both drugs. It may modify the diagnostic, preventive or therapeutic activity of either drug [1]. In poly pharmacy, it is important to determine the incidence and frequency of occurrence of drug interactions, which serious implications, in hospitalized patients. In addition, it is also important to find out agents that are most likely to produce hazardous interactions[2]. As per survey, the incidence of drug interaction ranges from 3 to 5% in patients taking a few drugs to 20% in patients receiving many drugs. According to a report that, the drug interaction may be fourth to sixth leading cause for death in United States[3, 4].

Repaglinide is an oral insulin secretagoue of the meglitinide class. This agent is a derivative of benzoic acid and its structure is unrelated to that of sulfonylureas. However, like sulfonylurea, Repaglinide stimulates insulin release by closing ATP-dependent potassium channels in pancreatic β cells[1]. Repaglinide is rapidly absorbed following oral administration, reaching peak concentrations 30–60 min post dosing. The drug has a fast onset and a relatively short duration of action. Thus, it can be taken just before main meals, enhancing the prandial insulin response and reducing postprandial glucose excursions, thereby improving overall glycemic control. The elimination rate of Repaglinide is very fast (plasma half-life [t-1/2] of ~1 h)[2, 3]. In patients with normal kidney function, the drug is almost completely (98%) metabolized in the liver and is excreted primarily through the bile. Only a very small fraction (<8%) of the administered dose is excreted through the urine[3-5]. None of its major metabolites contribute to the glucose-lowering effect and the drug does not appear

to accumulate with repeated dosing[6]. Since, Repaglinide has a short duration of action and is excreted independently of renal function, it may be suitable for use in patients with type-2 diabetes with renal impairment. However, two previous short-term clinical studies on the pharmacokinetics of Repaglinide in subjects with renal impairment showed some slight differences compared with patients with normal renal function[7,8]. One study showed that the area under the curve (AUC) and maximum serum concentration (Cmax) for Repaglinide were significantly higher in subjects with renal impairment than in healthy subjects, but were independent of the degree of renal impairment[9]. A second study showed that the elimination of repaglinide was slower in patients with severe renal impairment (creatinine clearance [CLCR] of 39–20 ml/min) than in those with normal or mild/moderately impaired renal function[10].

Very few plants widely used in folk medicine, are still tested and screened for their pharmacological activities. Yet they provide an unlimited source of big interest compounds which can further become new active drugs. Recently, there has been renewed interest in the use of plant compounds as antidiabetic compounds[11] and more than 1200 plant species have been found to exhibit antidiabetic properties[12, 13] and[14, 15]. Furthermore, the WHO expert committee recommended scientific investigation of hypoglycaemic agents of plant origin for the treatment of diabetes mellitus[16]. Mangiferin, is a naturally occurring glucosylxanthone, is an active phytochemical present in several traditionally used medicinal plants including *Mangifera indica Linn.* Family: Anacardiacae, Genus: Mangifera)[17,18] that has been shown to exhibit multiple pharmacological effects that include antioxidant[19], anti-inflammatory[20-22] and immunomodulatory activities[23,24]. Diminutions in glutamate-induced neurotoxicity and memory enhancement effects of mangiferin have also been reported[25,26]. Mango fruit is rich in mangiferin[27] and, according to Nadkarni[28], the ripe fruit is very wholesome, nourishing, and useful in nervous and atonic dyspepsia and constipation. Mangiferin is present in leaves[29], fruits, stem and roots[30] of *Mangifera indica*. The natural C-glucoside xanthone Mangiferin [2-C-β-D-gluco-pyranosyl-1,3,6,7- tetrahydroxyxanthone; C19H18O11 ; Mw., 422.35; melting point, anhydrous 271°C[31]. Mangiferin, has been reported to posses antitumor[32], antiviral[33] activities. Mangiferin rich plants are widely used medical plants in India for the treatment of immune-deficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders[34]. Being a polyphenolic antioxidant, Mangiferin has strong antioxidant, antilipid peroxidative, immunomodulatory, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities[35]. Mangiferin, has recently been shown to have antidiabetic activity in KK/Ay mice, a genetic model of non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia[17]. Mangiferin have been reported to be potent alpha-glucosidase inhibitors that have been shown to inhibit increases in serum glucose levels[25]. Mangiferin also shows modulation of certain P450 enzymes[37].

Since Repaglinide has the possibility of interacting with enzyme inducers or inhibitors of CYP3A4 and CYP2C8 and Mangiferin being a CYP450 enzyme modulator. This study is carried out to evaluate the effect of Mangiferin on the lipidprofile, hypoglycemic and hepatotoxicity activity of Repaglinide in experimental animals. As Repaglinide in known to cause hepatotoxicity they are contraindicated in patients with pre-existing liver disease. The toxic effects of these drugs in combination may be enhanced or protected. Hence in the present study we undertake the hepatotoxicity activity along with the parameters like Fasting blood sugar, TG, TC, LDL and HDL and hepatotoxicity studies will be carried out by measuring the AST, ALT levels to assess the extent of liver damage. All the six parameters will be done using the drugs individually and in combination. All studies will be carried out in rats.

MATERIALS AND METHODS

Plant extract, Chemicals and Drugs

Mangiferon (gift sample) was obtained from Sigma Aldrich, Bangalore, India. Repaglinide [Pharma grade](gift sample) from Micro Labs Pvt Ltd, HP, India. Glucose, HDL, Total cholesterol and Triglycerides estimation assay kits was purchased from, Pericugent, Thane, India. Alloxan was obtained from Sigma Aldrich, Bangalore, India. All other chemicals and reagents used in the study were of analytical grade.

Dose Selection

Repaglinide(0.2mg/kg) body weight by p.o route.

Mangiferin(200mg/kg) body weight by p.o route.

Animals

Male wistar albino rats weighing between 200-250gms were used for the experiments. Animals were housed for a week in the laboratory before the start of the experiment. Rats were fed with standard rodent diet (Lipton India Ltd) and water ad libitum. Ethical committee clearance was obtained prior from college ethical committee for the present study**.** (IAEC/NCP/62/11)

(Each group having 10 Wistar albino rats)

Group1: Vehicle control

Group2: Alloxan induced diabetic rats(150 mg/kg)

Group3: Alloxan + Repaglinide (oral)

Group4: Alloxan + Mangiferin (oral)

Group5: Alloxan + Repaglinide (oral) + mangiferin (oral)

Parameters: Fasting blood sugar, TG, TC, LDL and HDL

Anti-diabetic activity was done in rats. Rats received an intraperitoneal injection of Alloxan 150mg/kg of single dose, after 48hrs they were confirmed diabetic by estimating the blood glucose level[37]. Animals showing fasting blood glucose level 250 were considered diabetic. Animals were divided into four groups containing six animals each. The first group served as control whereas, the second group received Repaglinide(0.2mg/kg) , third group Mangiferin (200mg/kg) and fourth group received combination of Mangiferin(200mg) and Repaglinide(0.2mg/kg).Dosing was given for 10 days. And on 11th day, Blood samples were obtained by retro orbital flexes in order to measure the blood glucose level. The blood glucose level was estimated by trinder‟s method using semiautoanalyser. The instrument was calibrated by using the standard solution (Glucose 100mg/dl) from the kit.

Hepatotoxicity studies were done in rats. Animals were divided into four groups containing six animals each. The first group served as the control whereas the second and third group received Repaglinide(0.2mg/kg) and Mangiferin (200mg/kg) individually. The fourth group received a combination of Repaglinide (0.2mg/kg) and Mangiferin (200mg/kg). After weeks treatment with the drugs, the animals blood were removed by retro orbital plexus upto 2ml in the effendroff tube, the blood were allowed to clot at room temperature for 15 min. after that the serum is obtained by centrifugation at 3500 rpm for 15 min. soon after AST and ALT level in serum were measured by kinetic method using kit from ERBA diagnostics in Bioanalyser.

Statistical Analysis

Results are expressed as mean ± SEM. Statical analysis was performed using the Graph pad prism-5, Graph pad software, USA and “n” indicated the number of animals used. Differences between means were assessed by One-way analysis of variance (ANOVA) followed by Dunnet‟s ‘t’ test.

RESULTS

Influence of the drug interaction on the Ant diabetic activity was assessed, by studying the effects of the two drugs on the fasting blood sugar and lipid profile when administered individually and in combination over a period of 7 days. Comparisons were made with untreated Alloxan induced diabetic group. Forty eight hours after injection of Alloxan rats exhibited a significant rise in Fasting Blood Sugar levels (FBS) which was three to four folds higher than the normal values (Table 1). Repaglinide at a dose of 0.2mg/kg (p.o) significantly reduced the FBS level by 37.22% and Mangiferin at a dose of 200mg/kg (p.o). By 24.27% where as a reduction of 71.43% was observed when they were administered concomitantly. The extent of the reduction in FBS brought about by concomitant administration of Repaglinide and Mangiferin (71.43%) was significantly higher than that observed for the groups that were treated only with Repaglinide (37.22%) or Mangiferin (25.27%). The raised cholesterols a level (208.78 ± 8.58) of diabetic rats was significantly reduced by Repaglinide and Mangiferin when administered alone and concomitantly (Table-2). Decrease in cholesterol levels brought about by Repaglinide (137.42±9.45) and Mangiferin (148.98±9.99) when administered individually was not significantly different from that obtain, when they were administered concomitantly (129.22±6.16).

Alteration of the significantly raised triglyceride levels in diabetic rats (192.4±4.32) by Repaglinide (186.8±8.12), Mangiferin (192.4±4.32) and their combination (170.32±6.41) was not significant (Table no 2). There was no significant change in the level of HDL in the Alloxan induced diabetic rats (44.29±3.22) when compared with the control (54.12±5.16). Administration of Repaglinide, Mangiferin individually or in combination did not significantly alter the HDL levels (Table-3). The LDL levels in the Alloxan induced diabetic rats (164.22±7.04) were significantly higher than those observed in normal rats (68.45±2.11). These values were reduced significantly in the groups that were treated with Repaglinide (92.06±6.03), Mangiferin

(88.24± 9.02) and a combination of the two administered concomitantly (82.18±12.92). No significant difference in LDL values is observed when comparison is made between the groups treated with Repaglinide, Mangiferin and the group treated with a combination of both (Table-3). The VLDL levels in the Alloxan induced diabetic rats (39.42±0.953) was significantly higher than that observed in vehicle control groups(22.37±4.89).Diabetic groups treated with Repaglinide , Mangiferin and a combination of the two exhibit the significantly lower value of (25.03±1.46,23.43±2.19,24.54±2.68) as compare to control diabetic rats. No significant difference in VLDL values is observed when comparison is made between the groups treated with Repaglinide, Mangiferin and the group treated with a combination of both (Table-3).

Assessment of potential hepatotoxicity of Repaglinide and Mangiferin when administered individually and in combination was carried out by measuring the alterations in the serum SGOT and SGPT levels brought about by them in non diabetic rats treated with the drugs over a period of seven days. The Serum levels of ALT in the group treated with Repaglinide (99.68± 9.908) did not differ significantly with that observed in the control group (55.78±14.84). No significant alteration was observed when Repaglinide and Mangiferin were administered (48.81±90.07).concomitantly. Mangiferin administered alone also did not alter the ALT levels significantly. Comparison of serum levels of ALT between groups treated with Repaglinide and its combination with Mangiferin did not show a significant difference (Table-4).

Treatment with Repaglinide significantly elevated (160.4±7.848).the serum AST levels above the normal values observed in the control group (81.15± 6.394). Concomitant administration of Mangiferin significantly inhibited the rise in the value of AST brought about by Repaglinide. Mangiferin alone significantly reduced the serum AST levels (14.62±0.6631) to a value below that observed in normal rats (Table-5).

DISCUSSION

Mangiferin a xanthone glucoside has been reported to possess ant diabetic activity in kk/ay mice, a genetic model of NIDDM with hyper insulimia. It has also been reported to be a potent Alpha-glucosidase enzyme inhibitor and a modulator of certain CYP 450 enzymes that are involved in oxidative metabolism of wide variety of exogenous chemicals and endogenous compounds such as steroids, fatty acids and prostaglandins. Inhibition of ferrous citrate induced mitochondrial lipid peroxidation has also been reported.

There is indiscriminate use of herbal products containing Mangiferin as adjuvant in the treatment of diabetes. Enhanced side effects, toxic effects, or alterations in the effect of the main drug that necessitates dose corrections, may arise due to the interaction of Mangiferin with other drugs used in therapy. Self medication without medical supervision adds the possibility of consequence of such interactions going unnoticed until serious issues arise. Very few reports are available in the literature regarding interactions between ant diabetic agents and Mangiferin.

Repaglinide is an insulin secretagogue and is used as a drug in the treatment of diabetes in patients who require control of post-prandial glucose level. It has a short half life of one hour and is mainly metabolized in the liver by Cytochrome P450 enzyme system. Mangiferin is a common component of many herbal preparations used by diabetics. It is also known to be metabolized by the Cytochrome P450 enzyme system and modulate the activity of certain enzymes that belong to the system. The current study was carried out as a preliminary investigation into possible alterations in the (FBS) and lipid profile that can arise due to drug interaction when the two drugs are administered concomitantly. As both the drugs are metabolized in the liver the study was extended to observe for toxicity in the normal liver.

Repaglinide significantly reduced the elevated (FBS) level in diabetic rats by 37.22% and Mangiferin by 24.27% when administered individually, whereas concomitant administration reduced the value by 71.43%. Repaglinide is known to be an insulin secretagogue that acts by its action on the β cells whereas Mangiferin is an α -glucocidase inhibitor and acts by inhibition of glucose absorption and also by decreasing insulin resistance. Higher reduction in (FBS) levels when the two drugs were administered concomitantly therefore appears to be more due to nonlinear effect rather than due to any interaction between them. Serum triglycerides are significantly elevated in the Alloxan induced diabetic group. Repaglinide alone or in combination with Mangiferin was unable to significantly alter the raised triglyceride levels observed in diabetic rats. Further there was no significant difference in the triglyceride levels observed between the group that was

administered Repaglinide and the one in which it was administered in combination with Mangiferin. Triglyceride levels of the group administered Mangiferin did not significantly differ from that observed in diabetic group. Although Mangiferin has been reported to reduce the triglyceride levels in adiposities, its ability to reduce the serum triglyceride level is insignificant when administered alone or in combination with Repaglinide.

Total cholesterol, LDL and VLDL are significantly elevated in the diabetic control group as compared to the non diabetic group. Significant reduction in the levels of the same was observed on administration of Repaglinide or Mangiferin or a combination of the two. There was no significant difference in the serum levels of the above parameters when comparisons were made between the groups treated with Repaglinide, Mangiferin or a combination of both. Repaglinide by its ability to act as an insulin secretagogue is reported to reduce the elevated levels of cholesterol, LDL and VLDL. Possible drug interaction between Mangiferin and Repaglinide if any does not alter the positive effects observed with the administration of Repaglinide. There was no significant alteration of HDL levels in the groups treated with Repaglinide, Mangiferin or a combination of both when compared with the diabetic control.

There was no significant alteration of serum ALT levels in the groups treated with Repaglinide, Mangiferin or a combination of both when compared with normal control. Serum AST levels were significantly elevated in the Repaglinide treated group whereas significantly decreased below normal in Mangiferin treated group as compared to the levels observed in the normal control. Concomitant administration of both significantly decreased the elevated values observed when, only Repaglinide was administered.

CONCLUSION

Mangiferin is a popular ingredient in many herbal preparations that are used as adjuvant along with anti diabetic drugs. Very few reports are available regarding its interactions with the main drugs that are in use for glycemic control. From the current study, the outcome of a combination of Mangiferin with Repaglinide in the treatment of type 2 diabetics can be concluded that a combination of Repaglinide and Mangiferin lowers the FBS by a value that is much below attained by administration of Repaglinide. It does not alter the hypocholestremia brought about by Repaglinide and also its ability to reduce the elevated LDL and VLDL levels observed in diabetes**.** It does not alter the serum ALT levels on its own. On use in combination with Repaglinide elevated AST levels associated with Repaglinide are significantly reduced when the two drugs are used concomitantly. Magiferin can be safely used as an adjuvant in the treatment of diabetes as it does not adversely alter the beneficial effects produced by the use of Repaglinide even when it is administered in combination with Repaglinide. Its ability to decrease insulin resistance and modulate the CYP450 enzyme system responsible for the metabolism of Repaglinide can be utilized in treatment with necessary corrections in the dose, to avoid hypoglycemia.

Table 1: Table showing the influence of repaglinide and mangiferin on fasting blood sugar in alloxan induced diabetic rats

PARAMETERS	DAYS	GROUP 1 VC	GROUP 2 ALLOXAN 150mg/kg	GROUP 3 RPG 0.2mg/kg	GROUP 4 MGF 200mg/kg	GROUP 5 RPG+MGF 0.2mg/kg+200mg/kg
GLUCOSE (mg/dl)	0	124.0±14.73	251.2±13.09	262.8±12.41	242.2±14.62	403.8±8.73
	7	102.4±5.34	241.7±11.42	163.3±6.05	174.8±5.92	115±1.76
% REDUCTION IN GLUCOSE		16.95±5 **	3.79±3.4a	37.22±3.3 **b	25.27±6.27 ** b	71.43±9 **b
N	5	5		6	6	6

a=compared with vehicle control
b=compared with alloxan
N=No. of Animals
Values are expressed as mean ± SEM
***=p<0.001, **=p0.01, *=p<0.05

Table 2: Table showing the influence of repaglinide and mangiferin on lipid profile in alloxan induced diabetic rats

PARAMETERS	DAYS	GROUP 1 VC	GROUP 2 ALLOXAN 150mg/kg	GROUP 3 RPG 0.2mg/kg	GROUP 4 MGF 200mg/kg	GROUP 5 RPG+MGF 0.2mg/kg+200mg/kg
TG (mg/dl)	0	91.18±6.29	208.42±8.12	197.42±7.22	201.52±5.77	188.52±9.22
	7	88.14±2.19	197.14±6.81	182.85±8.12	192.4±4.32	170.32±6.41
		P<0.01**		P>0.05	P>0.05	P>0.05
TC (mg/dl)	0	120.49±2.06	198.92±6.15	208.53±11.62	205.46±4.96	213.4±9.13
	7	134.52±12.33	208.78±8.58	137.42±9.45	148.98±9.99	129.22±6.16
		P<0.01**		P>0.01**	P>0.01	P>0.01

a=compared with vehicle control
b=compared with alloxan
N=No. of Animals
Values are expressed as mean ± SEM
***=p<0.001, **=p<0.01, *=p<0.05

Table 3: Table showing the influence of repaglinide and mangiferin on lipid profile in alloxan induced diabetic rats

PARAMETERS	DAYS	GROUP 1 VC	GROUP 2 ALLOXAN 150mg/kg	GROUP 3 RPG 0.2mg/kg	GROUP 4 MGF 200mg/kg	GROUP 5 RPG+MGF 0.2mg/kg+200mg/kg
HDL(mg/dl)	0	48.99±.42	40.41±1.91	34.48±1.26	48.65±.94	44.66±3.65
	7	54.12±5.16	44.29±3.22	41.29±6.12	52.51±3.39	49.65±2.91
		P>0.05		P>0.05	P>0.05	P>0.05
LDL(mg/dl)	0	52.04±8.11	144.12±3.12	138.72±12.32	146.74±13.21	144.12±8.42
	7	68.45±2.11	164.22±7.04	92.06±6.03	88.24±9.02	82.18±12.91
		P<0.01**		P<0.01**	P<0.01**	P<0.01**
VLDL(mg/dl)	0	10.12±2.13	14.34±3.45	9.46±2.38	11.25±2.14	10.98±4.21
	7	22.37±4.89	39.42±.953	25.03±1.46	23.43±2.19	24.54±2.68
		P<0.01**		P<0.01**	P<0.01**	P<0.01**

a=compared with vehicle control
b=compared with alloxan
N=No. of Animals
Values are expressed as mean ± SEM
***=p<0.001, **=p0.01, *=p<0.05

Table 4: Effect on serum ALT level after 7 days of administration of repaglinide and mangiferin to normal rats

DOSE AND ROUTE GROUPS	GROUP 1 VEHICLE CONTROL	GROUP 3 REPAGLINIDE 0.2mg/kg	GROUP 4 MANGIFERIN 200mg/kg	GROUP 5 REPAGLINIDE + MANGIFERIN 0.2mg/kg+200mg/kg
SERUM ALT LEVELS (U/L) MEAN± SEM	55.78±14.84	99.68±9.908 a	46.39±16.51 a	48.81±19.07 a
		P>0.05	P>0.05	P>0.05

a=compared with vehicle control
N=No. of Animals
Values are expressed as mean ± SEM
***=p<0.001, **=p<0.01, *=p<0.05

Table 5: Effect on serum AST level after 7 days of administration of repaglinide and mangiferin to normal rats

DOSE AND ROUT GROUPS	GROUP 1 VEHICLE CONTROL	GROUP 3 REPAGLINIDE 0.2mg/kg	GROUP 4 MANGIFERIN 200mg/kg	GROUP 5 REPAGLINIDE + MANGIFERIN 0.2mg/kg+200mg/kg
SERUM AST LEVELS (U/L) MEAN± SEM	81.15±6.397	160.4±7.848 a	14.62±0.6631 a	98.33±6.995 a
		P<0.01**	P<0.01**	P<0.01**

a=compared with vehicle control
N=No. of Animals
Values are expressed as mean ± SEM
***=p<0.001, **=p<0.01, *=p<0.05

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