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INFLUENCE OF DIOSGENIN ON PHARMACOKINETICS AND PHARMACODYNAMICS OF REPAGLINIDE IN RATS

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ABSTRACT

Introduction: *Antidiabetic herbal preparations are mostly used as an add-on therapy in diabetes mellitus and such type of herbal preparations often contain diosgenin. Hence, in the present work the effects of diosgenin on the pharmacodynamics and pharmacokinetics of repaglinide in both normal and streptozotocin (STZ) induced diabetic rats was studied.* ***Methods:*** *For pharmacokinetic and pharmacodynamic study all the rats were randomly divided into eight groups (n=6). First four groups represent normal rats and remaining four groups represents diabetic rats. Group I and V orally administered with repaglinide, group II and VI orally administered with diosgenin, group III and VII orally administered with diosgenin and repaglinide for single dose interaction study and group IV and VIII orally administered with diosgenin for 7 days and on 8th day with diosgenin followed by repaglinide for multiple dose interaction study. Blood samples were collected at different time intervals and used for further analysis.* ***Results:*** *In both normal and STZ induced diabetic rats the combination of repaglinide with diosgenin increased all the pharmacokinetic parameters including C_{max}, AUC_{0-n}, AUC_{total}, $AUMC_{total}$, MRT and $t_{1/2}$. And decreased the clearance, Vd when compared to control group. The combination of repaglinide and diosgenin improved the antidiabetic activity.* ***Conclusion:*** *The results showed that the combination of repaglinide and diosgenin led to the enhancement of absorption of repaglinide by inhibiting the CYP3A4 microsomal enzyme, which suggested that diosgenin might be beneficial as an adjuvant to repaglinide in a proper dose, in diabetic patients.*

KEY WORDS

CYP3A4, Diosgenin, Pharmacodynamic, Pharmacokinetic, Repaglinide

INTRODUCTION:

A combination of herbal drugs or isolated phytochemicals is found to be beneficial in some diseases when given along with allopathic drugs (Sandhya *et al*., 2012). Repaglinide is a meglitinide oral antidiabetic agent which is widely used in the treatment of type 2 diabetes mellitus. It produces the antihyperglycemic effect primarily by stimulating the insulin release by closing ATP-dependent K^+ channels in pancreatic β cells (Kirankumar *et al*., 2013). The antihyperglycemic effect of repaglinide can be altered by combining with other drugs. Eg: Mangiferin (Kirankumar *et al*., 2013). Thus, there is need to study the interaction between repaglinide and diosgenin to avoid adverse effects.

Diosgenin is a naturally occurring steroid saponin found abundantly in yams and legumes (Jayadev *et al*., 2012). Diosgenin is used in a variety of diseases, like cancer (Jayadev *et al*., 2012; Raju *et al*., 2009), diabetes (Mirunalini *et al*., 2011), cardiovascular disease, oxidative stress (Chih *et al*., 2015), and inflammation (Scott *et al*., 2015). Various studies proved that diosgenin is generally well tolerated orally up to 500 mg/kg doses (Scott *et al*., 2015). It is used as a starting material for the synthesis of steroidal drugs, because it exhibits estrogenic activity. It has received more attention because of the variety of their promising pharmaceutical properties (Mirunalini *et al*., 2011).

The main reason for drug interactions is inhibition or induction of microsomal CYP enzymes. Most of the herbal medicines can alter the bioavailability and

clearance of co-administered drugs by modifying hepatic and intestinal CYP enzymes (Sandhya *et al*., 2012). Diosgenin is metabolized by inhibition of CYP3A4 enzyme (Manda *et al*., 2013). Repaglinide is completely metabolized by CYP3A4 and CYP2C8 microsomal liver enzymes (Kirankumar *et al*., 2013).

The main aim of the present study was to estimate the influence of diosgenin on the pharmacokinetics and pharmacodynamics of repaglinide in normal and STZ induced diabetic rats to study the synergistic effects of concomitant treatment with these drugs.

MATERIALS AND METHODS

Animals and diet:

Male albino wistar rats weighing 200-280 g were purchased from Sainath Agencies, Hyderabad, India and used for this study after obtaining permission from the institutional animal ethical committee (CPCSEA Reg. No. IAEC/07/UCPSc/KU/2016). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light/dark cycle, at an ambient temperature of 25±5 °C, 35-60 % relative humidity). The animals were fed with standard rat pellet diet purchased from Vyas Labs, Hyderabad and water *ad libitum*.

Drugs and chemicals:

Repaglinide and Ritonavir (IS) were obtained as gift samples from Novartis, Hyderabad, India. Diosgenin was purchased from Yucca Enterprises, Mumbai, India. Streptozotocin was purchased from Sigma-Aldrich, Bangalore, India. Glucose estimation kit was purchased from Adi diagnostics, Hyderabad, India. HPLC grade methanol was purchased from Merck, Mumbai, India. Water used for analytical purpose was double distilled, filtered by using direct-Quv Millipore and sonicated for removing air bubbles. All other chemicals used for study were of analytical grade.

HPLC analysis of repaglinide:

A slightly modified reverse phase HPLC method (Seema *et al*., 2014) was used for the estimation of serum repaglinide concentration and quantified by a validated ultra-fast liquid chromatography coupled with photodiode array detection (Shimadzu Corporation, Kyoto, Japan). The system consisted of binary LC-20AD pumps with a micro gradient mixer. RP C18 column, (250 mm×4.6 mm, 5 µm, Phenomenex Luna) was used. By lab solutions software all the operations and analysis of data obtained were controlled. A mixture of methanol and distilled water in the ratio of 80:20 (v/v) used as mobile phase at a flow rate of 1 ml/min. By using sonicator the mobile phase was degassed and filtered through 0.22 µm membrane filter. Then detection was carried out at a wavelength of 260 nm. The total run time for analysis was 10 min.

100 µl of test rat serum was taken in centrifuge tubes, and then add 100 µl of internal standard (10 µg/ml) solution and vortex for 1 min. Then add 100 µl of methanol to precipitate the proteins. The mixture was vortexed for 1 min and centrifuged for 20 min at 3000 rpm. Clear supernatant was collected in another centrifuge tubes. 20 µl of aliquot was injected into the analytical column for analysis.

Intraday and inter day precision were obtained from three levels of quality control samples of repaglinide. The precision and accuracy of the method was determined by taking quality control samples at three concentrations of 2, 10, and 100 µg/ml and all the samples were run in three replicates. Intraday precision data was obtained in a single day by analyzing three sets of quality control samples, while the inter day data was obtained on three consecutive days by analyzing the quality control samples. The assay procedure was found to be precise and accurate. Limit of detection (LOD) and limit of quantification (LOQ) of repaglinide was found to be 0.287374 µg and 0.957912 µg respectively. Hence the LOD and LOQ were found to be within the range of the analyzed levels in serum samples (Ravikanth *et al*., 2011).

EXPERIMENTAL DESIGN

Pharmacokinetic interaction study in normal rats:

After overnight fasting, rats were randomly divided into four groups (n=6). Group I administered orally with repaglinide, 0.2 mg/kg (Chitra *et al*., 2009), Group II administered orally with diosgenin, 20 mg/kg (Kanchan *et al*., 2016), Group III administered orally with diosgenin (20 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for single dose interaction study and Group IV administered orally with diosgenin (20 mg/kg PO) for 7 days and on 8th day diosgenin (20 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) for multiple dose interaction studies.

Blood samples were collected through retro orbital plexus and immediately same volume of normal saline was replaced intra peritoneally. The blood was collected at time intervals of 0, 0.5, 1, 2, 4, 8, and 24 hrs in every group. Serum was separated after centrifugation at 8000 rpm for 15 min and the samples were stored at – 20 °C until analysis (Sandhya *et al*., 2012).

Pharmacokinetic interaction study in diabetic rats:

Induction of diabetes

Male albino wistar rats (200-280 g) were randomly selected and fasted overnight for study and diabetes was induced by intra peritoneal injection of streptozotocin 55 mg/kg body weight, in freshly prepared citrate buffer (pH 4.5) (Sandhya *et al*., 2012). After 72 hrs, blood samples were collected from rats by retro orbital plexus and the serum was analyzed for glucose levels by oxidase- peroxidase (GOD) method (Trinder, 1969). The animals with blood glucose level >250 mg/dl were considered as diabetic and were used in the study.

Grouping of diabetic rats:

Diabetic rats were divided into 4 groups (n=6) and were treated same as mentioned in normal rats. Blood samples were collected through retro orbital plexus by using heparinised capillary tubes, and immediately same volume of normal saline was replaced intra peritoneally. The blood was collected at time intervals 0, 0.5, 1, 2, 4, 8, and 24 hrs in every group. Serum was separated after centrifugation at 8000 rpm for 15 min and the samples were stored at – 20 °C until analysis (Sandhya *et al*., 2012).

Pharmacodynamic interaction study in normal and diabetic rats:

All the rats were divided in to 8 groups (n=6), 4 groups represent normal rats and remaining 4 groups represents diabetic rats. The effect of diosgenin, repaglinide and their combinations on fasting blood glucose level was studied up to 24 hrs. Blood samples were collected through retro orbital plexus by using heparinised capillary tubes at 0, 0.5, 1, 2, 4, 8, and 24 hrs after the treatment. The samples were analyzed for blood glucose using oxidase-peroxidase method (Trinder, 1969).

Statistical analysis:

All the results were expressed as mean ± SD. The data were statistically evaluated using one-way ANOVA with Bonferroni post-test performed using Graph Pad Prism 7.01 software. Values corresponding to $p<0.05$ were considered as significant.

RESULTS

Pharmacokinetic interaction study of diosgenin and repaglinide combination in normal rats:

The pharmacokinetic parameters in repaglinide treated group were calculated and showed C_{max} of 3.778±0.532 µg/ml, T_{max} of 1 hr and AUC_{0ton} of 23.06±3.184 µg.hr/ml in normal rats. Increase in C_{max}, AUC, and AUMC; and decrease in clearance and Vd was observed in single dose and multiple dose diosgenin pretreated groups and was statistically significant when compared with repaglinide alone group. All the pharmacokinetic parameters and mean concentration-time profile of normal rats were shown in Table 1 and Figure 1.

Table 1: Mean pharmacokinetic parameters of repaglinide in different groups of normal rats

PK Parameter	Repaglinide	Repaglinide+DSG (SDI)	Repaglinide+DSG (MDI)
C_{max} (µg/ml)	3.78±0.53	8.53±1.21**	9.99±2.93**
T_{max} (h)	1±0	1.16±0.41	1.16±0.41
AUC_{0-n} (h µg/ml)	23.06±3.18	46.37±15.34*	47.93±15.71*
AUC_{total} (h µg/ml)	23.18±3.14	49.91±17.92*	53.73±19.68*
$AUMC_{total}$	133.95±21.57	432.14±270.77	537.01±320.45
$t_{½}$ (h)	3.05±0.51	5.92±2.09*	6.98±2.78*
MRT (h)	5.77±0.40	8.29±2.34	9.07±3.42
CL (ml/h/kg)	9.13±1.73	2.87±1.34	2.23±1.61
Vd (ml/kg)	39.62±6.31	21.41±3.36	17.87±4.65

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

*p<0.05; **p<0.01 considered as significant when compared with repaglinide control. DSG- diosgenin, SDI- single dose interaction, MDI- multiple dose interaction

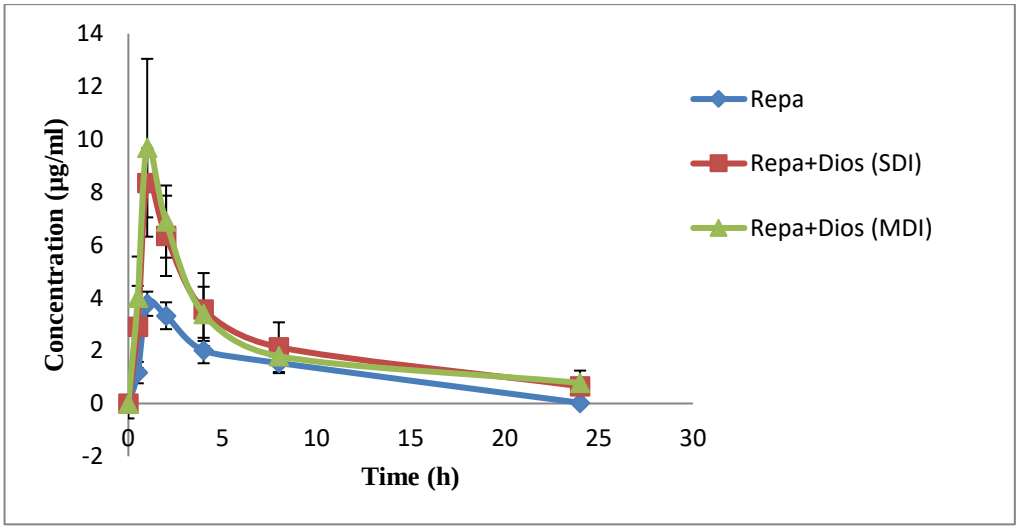

Figure 1: Mean serum concentration – time profile of repaglinide in normal rats

Pharmacokinetic interaction study of diosgenin and repaglinide combination in STZ induced diabetic rats: Pharmacokinetic parameters of repaglinide alone group in diabetic rats were calculated and showed C_{max} of 7.398±0.655 µg/ml, T_{max} of 1.16±0.408 hr and AUC_{0ton} of 41.393±6.355 µg.hr/ml. C_{max}, AUC, and AUMC were increased in single dose and multiple dose diosgenin pretreated groups. Clearance and Vd was decreased and is statistically significant in single dose and multiple dose diosgenin treated groups when compared with repaglinide alone group. All the pharmacokinetic parameters and mean concentration-time profile of diabetic rats were shown in Table 2 and Figure 2.

Table 2: Mean pharmacokinetic parameters of repaglinide in different groups of diabetic rats

PK Parameter	Repaglinide	Repaglinide+DSG (SDI)	Repaglinide+DSG (MDI)
C_{max} (µg/ml)	7.39±0.65	13.07±1.11**	19.01±2.21***
T_{max} (h)	1.16±0.41	1.33±0.51	1.33±0.51
AUC_{0-n} (h µg/ml)	41.39±6.35	69.15±10.42**	90.51±13.74***
AUC_{total} (h µg/ml)	42.39±6.54	73.64±13.58*	99.52±17.98**
$AUMC_{total}$	267.12±95.98	596.45±240.50	916.76±345.25**
$t_{½}$ (h)	3.91±1.69	5.69±1.59*	6.95±1.82*
MRT (h)	6.20±1.83	7.82±1.86	8.96±2.08*
CL (ml/h/kg)	4.57±1.69	1.68±0.53	1.01±0.22
Vd (ml/kg)	22.99±2.37	12.92±1.73	9.74±0.72

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

*p<0.05; **p<0.01; ***p<0.001 considered as significant when compared with repaglinide control. DSG- diosgenin, SDI- single dose interaction, MDI- multiple dose interaction

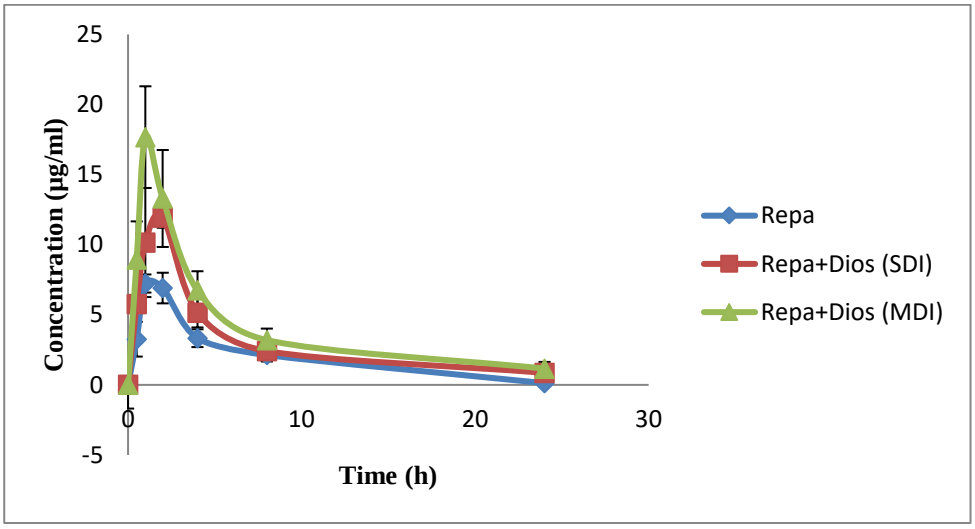

Figure 2: Mean serum concentration – time profile of repaglinide in diabetic rats

Pharmacodynamic interaction study of diosgenin and repaglinide combination in normal rats:

At each time point the mean blood glucose levels were calculated using glucose oxidase peroxidase method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated in normal rats. The mean blood glucose levels and percentage glucose reduction was compared against repaglinide alone group in normal rats. Diosgenin pretreated groups were found to have significant effect on the diabetic control and the percentage reduction of glucose levels in normal rats. Mean blood glucose levels of normal rats were shown in Figure3.

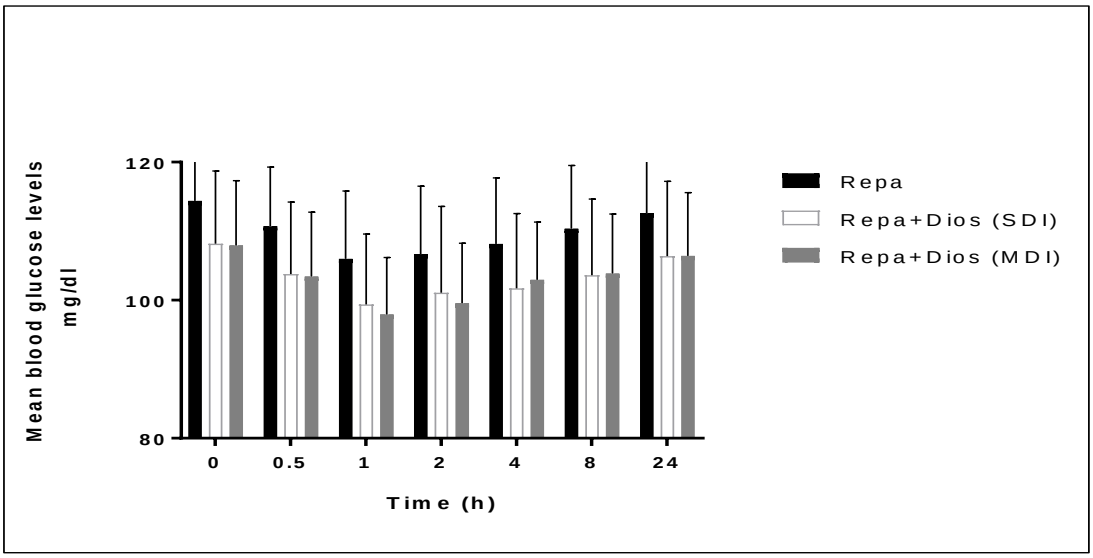

Figure 3: Effect of pretreatment of diosgenin and repaglinide on blood glucose levels of normal rats

Pharmacodynamic interaction study of diosgenin and repaglinide combination in diabetic rats:

At each time point the mean blood glucose levels were calculated using glucose oxidase peroxidase method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated in diabetic rats. The mean blood glucose levels and percentage glucose reduction was compared

against repaglinide alone group in diabetic rats. Diosgenin pretreated groups were found to decrease the mean blood glucose levels and thus increase the percentage glucose reduction in both single dose and multiple dose exposure, with more statistical significance in multiple dose group $p < 0.05$. Mean blood glucose levels of diabetic rats were shown in Figure 4.

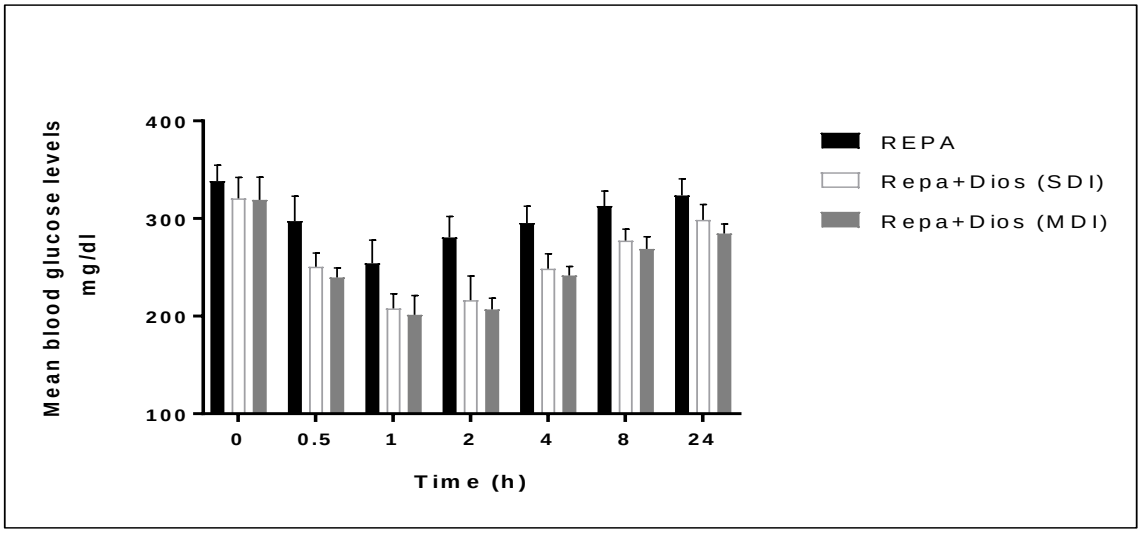

Figure 4: Effect of pretreatment of diosgenin and repaglinide on blood glucose levels of diabetic rats

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder resulting from a relative deficiency of insulin secretion and/or insulin resistance. The main aim of this study is to determine the influence of diosgenin on the pharmacokinetics and pharmacodynamics of repaglinide in normal as well as in STZ induced diabetic rats. The main reason for interaction is either by induction or inhibition of CYP microsomal enzymes (Prasad *et al*., 2012). Increase in the plasma concentration of simultaneously administered drugs enhances the pharmacological and toxicological effect by inhibition of CYP enzymes. Decrease in the plasma concentration of simultaneously administered drugs; reduce the therapeutic effect due to induction of CYP enzymes (Prasad *et al*., 2012). Herbal drugs which alter hepatic and intestinal CYPs can modify the bioavailability and clearance of administered drugs. Repaglinide is completely metabolized by CYP3A4 and CYP2C8 microsomal enzymes (Kirankumar *et al*., 2013). Diosgenin is metabolized by CYP3A4 microsomal enzyme (Manda *et al*., 2013). In this study, we determine the influence of diosgenin on the pharmacokinetics and pharmacodynamics of repaglinide in normal and STZ induced diabetic rats. We found that pharmacokinetic parameters were changed in diosgenin pretreated groups, but statistically more significant in multiple dose treated group. In normal rats, diosgenin treated groups showed AUC_{total} 53.732±19.681 ($p<0.05$) and $AUMC_{total}$ 537.01±320.45. In STZ induced diabetic rats, diosgenin pretreated groups showed AUC_{total} 99.518±17.984 ($p<0.01$) and $AUMC_{total}$ 916.766±345.25 ($p<0.01$). There is increase in the T_{max} in both normal and STZ induced diabetic rats, may be due to change in the rate of absorption. The alteration in the metabolism of repaglinide may be due to, either by enhancing absorption or by inhibiting CYP3A4 enzyme which is responsible for metabolism of repaglinide.

We also found there is significant effect on the antihyperglycemic effect or the percentage reduction of glucose levels in diosgenin pretreated normal rats. At 1 hr we observed maximum percentage glucose reduction in all the groups of normal rats. In STZ induced diabetic rats, we found decrease in mean blood glucose levels significantly and increase in the percentage of glucose reduction at 1 hr in repaglinide alone, single dose and multiple dose diosgenin pretreated groups

(24.93±5.07%, 35.76±4.35% and 37.99±6.73% respectively). Percentage of glucose reduction was much significant ($p<0.01$) in multiple dose than in single dose treated groups ($p<0.01$).

Increase in the AUC and AUMC in diosgenin pretreated groups suggests that there is an inhibitory effect of diosgenin on intestinal metabolism of repaglinide as diosgenin has poor bioavailability (Jayadev *et al*., 2012). The influence of diosgenin was more in STZ induced diabetic rats by enhancing glycemic control, partly by increased pharmacokinetics of repaglinide and partly by antihyperglycemic activity of diosgenin.

There was more significant effect on the percentage glucose reduction in diabetic rats under multiple dose diosgenin treatment ($p<0.01$) and less significant effect in normal rats ($p<0.05$). Therefore, we observed the increased pharmacokinetic parameters of repaglinide more in multiple dose diosgenin pretreated groups and the pharmacodynamic activity was improved significantly only in STZ induced diabetic rats in multiple dose diosgenin pretreated group.

CONCLUSION

The results showed that repaglinide level was increased possibly by the metabolic inhibition of CYP3A4 enzyme in the presence of diosgenin. The combination of diosgenin with repaglinide considerably enhances the glucose lowering effect of repaglinide. Hence, the dose of repaglinide may require a special attention if used along with diosgenin containing herbal preparations to avoid complications.

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