

Assessment of Pharmacodynamic and Pharmacokinetic Interaction of Aqueous Extract of *Cassia auriculata* L. and Metformin in Rats

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ABSTRACT

Background: *Cassia auriculata* L. (CA) leaf extract might increase the body's production of insulin thereby suppressing the elevated blood glucose and lipid levels in diabetic rats. CA has been used as dietary supplement in India from ancient times. **Objective:** The present study was to elucidate the synergistic pharmacodynamic (PD) and pharmacokinetic (PK) interaction of metformin (MT) with CA. **Materials and Methods:** A simple, precise reverse phase high performance liquid chromatography - UV detection mode method was developed to quantify MT in rat plasma. In PD interaction, streptozotocin (45mg/kg, intraperitoneally) induced diabetic Wistar rats weighing 180–250 g of either sex were randomized to receive MT (90 mg/kg, MT-HD), CA (500 mg/kg) separately and in combination of MT (90 mg/kg, MT-HD) + CA (500 mg/kg), and MT (45 mg/kg, MT-LD) + CA (500 mg/kg) (all oral) along with normal and diabetic control groups for 21 days. PK of MT was carried out in normal rats with preadministration of CA (500 mg/kg) for 14 days. **Results:** PD data showed reasonable blood glucose lowering effect of CA. The reduction of MT dose with combination of CA achieved a similar blood glucose lowering effect of MT alone. PK data showed enhanced time taken to achieve maximum plasma concentration (C_{max}), area under the curve (AUC_{0-t}), and C_{max} in combination group of MT (90mg/kg) and CA (500mg/kg), and reduction of MT dose in Group III had a reduced C_{max} and AUC_{0-t} compared to MT alone treated groups. **Conclusion:** Co-administration of CA with MT at varying dose showed a synergistic herb-drug interaction. Thus using the synergistic herb-drug interaction, the dose level of MT may be reduced to produce the same therapeutic effect as when taken alone.

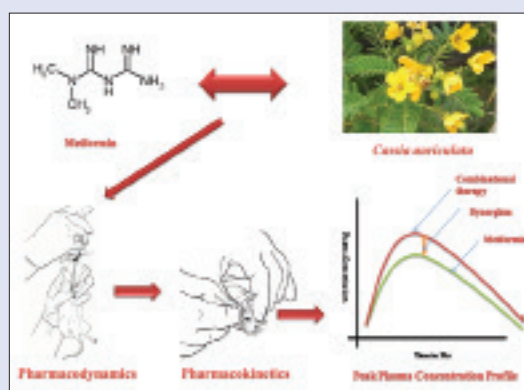
Key words: *Cassia auriculata* L., diabetes mellitus, herb-drug interactions, metformin, pharmacodynamic, pharmacokinetics

SUMMARY

- Elucidating the synergistic pharmacodynamic (PD) and pharmacokinetic (PK) interaction of metformin (MT) with *Cassia auriculata* L. (CA)
- PD data showed reasonable blood glucose lowering effect of CA. The reduction of MT dose with combination of CA achieved a similar blood glucose lowering effect of MT alone
- PK data showed enhanced time taken to achieve maximum plasma concentration (C_{max}), area under the curve (AUC_{0-t}), and C_{max} in combination

group of MT and CA, and reduction of MT dose had a reduced C_{max} and AUC_{0-t} compared to MT alone treated groups

- Co-administration of CA with MT at varying dose showed a synergistic herb-drug interaction. Thus using the synergistic herb-drug interaction, the dose level of MT may be reduced to produce the same therapeutic effect as when taken alone.



Abbreviations used: CA: *Cassia auriculata* L., PD: Pharmacodynamic, PK: Pharmacokinetic, MT: Metformin, RP-HPLC-UV: Reverse phase High Performance Liquid Chromatography-UV detection mode, T_{max} : Time taken to achieve maximum plasma concentration, AUC_{0-t} : Area under the curve, C_{max} : Maximum plasma concentration.

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DOI : 10.4103/0973-1296.168986

Access this article online

Website: www.phcog.com

Quick Response Code:



INTRODUCTION

Cassia auriculata L. (CA) is profoundly used as a tonic, astringent, antidiabetic, and conjunctivitis and ophthalmic in Ayurvedic medicine.^[1] It is one of the principle constituent of Aavaari panchanga chooranam; an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level.^[2-4]

CA is a much branched shrub with smooth cinnamon brown bark and closely pubescent branchlets. It has alternate, stipulate, glabrous, and slender leaves. Flowers are irregular, bisexual, bright yellow, and flowering throughout the year. Fruit is a short legume, 7.5–11 cm long, oblong, obtuse, flat, thin, and pale brown. Fruiting occurs throughout the year. 12–20 seeds per fruit are carried in its separate cavity.

Many studies have suggested the antidiabetic effect of CA which includes

in restoring the blood lipids and proteins to normal level as compared to standard drug phenformin.^[3] Studies have concluded the safety profile of CA for anti-hyperglycemic and hypoglycemia activities.^[4,5] The mechanism through which the CA could act is due to its insulinogenic

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Cite this article as: Elango H, Ponnusankar S, Sundaram S. Assessment of Pharmacodynamic and Pharmacokinetic Interaction of Aqueous Extract of *Cassia auriculata* L. and Metformin in Rats. Phcog Mag 2015;11:S423-6.

action. In addition, altered glucose homeostasis improved by feeding the extract through amelioration in the carbohydrate metabolic pathways.^[6]

Diabetes mellitus, caused by an absolute or relative deficiency of insulin or its function, lead to number of complications; it is emerging as the factor responsible for chronic disability and even death. Most of the secondary failures are observed with monotherapy and devastating long-term consequence of poor glycemic control. Furthermore, it is always beneficial to switch over the patient on combination therapy. A reasonable goal of the treatment is to maintain good glycemic control through combination therapy to keep the blood glucose level to near normal to a particular patient.^[7]

The first choice of biguanide for the recently diagnosed diabetic patients is metformin (MT). It acts by reducing hepatic glucose production and improving insulin sensitivity. Studies have suggested that administration of MT with phytoconstituents/herbal products presumably enhanced therapeutic effects;^[8] however, sufficient data is lacking with respect to their potential pharmacodynamic (PD) and pharmacokinetic (PK) interaction. Added exploration on their interaction between CA and MT at PK-PD levels will provide conceivable interest in administering in combination for the treatment of diabetes.

MATERIALS AND METHODS

Chemicals and reagents

MT was purchased from Cipla Pharmaceuticals (Mumbai, India). Streptozotocin from Hi-Media Laboratories Pvt. Ltd., (Mumbai, India). High performance liquid chromatography (HPLC) grade acetonitrile was procured from Rankem, New Delhi. Analytical grade potassium dihydrogen orthophosphate was obtained from Qualigen fine chemicals; Thermo Electron LLS India Pvt Ltd. Water for HPLC was purchased from Nice Chemicals Pvt Ltd., Kochi, Laboratory grade orthophosphoric acid was obtained from SD Fine Chem. Ltd., Mumbai.

Quality control of *Cassia auriculata* L. extract

The CA extracts were procured from Green Chem. Herbal Extracts, Bangalore, India, which were collected during the month of March and authenticated by Quality Assurance Department of Green Chem Herbal Extracts, Bangalore, India. The Certificate of Analysis was received and preserved as authentication record for further correspondence.

Animals

The experimental design for animal study was approved by Institutional Animal Ethics Committee (IAEC) of JSS College of Pharmacy, Udhagamandalam. Adult albino rats of Wistar strain (180–240 g) of either sex were housed at 12 h light/dark cycle at room temperature (27°C ± 2°C) and fed for 24 h with food and water *ad libitum*. The approved protocol (JSSCP/PhD/PHCHEM/06/2012–13) was conducted in accordance with guidelines as per “Guide for the care and use of laboratory animals.”

Preparation of *Cassia auriculata* L. extract

Dried powder of CA were extracted with boiling distilled water for 2 h evaporated to dryness and kept at 4°C yield of aqueous extract was found to 15%.

Phytochemical analysis of *Cassia auriculata* L. extract

The extract was subjected to preliminary phytochemical analysis to detect the presence of different phytochemicals. The aqueous extract was subjected to qualitative analysis for the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins, and sterols using the standard methods.

Induction and assessment of diabetes for pharmacodynamic study

The overnight fasted adult albino rats of Wistar strain weighing 180–240 g were induced diabetes by single intra-peritoneal injection of streptozotocin (45 mg/kg) prepared by dissolving in citrate buffer (0.1 M pH 4.4). The fasting glucose levels were assessed using one touch glucometer (Accu-Chek Active). The inception value of fasting glucose level to diagnose diabetes was considered to be more than 250 mg/dL after 48 h and used for PD study.

Experimental pharmacodynamic study design

Adult albino Wistar rats were divided into six groups each consisting of six animals as follows:

Group 1: Normal Control Group, 2: Diabetic Control Group, 3: MT (90 mg/kg), Group 4: CA (500 mg/kg), Group 5: MT (90 mg/kg) + CA (500 mg/kg), and Group 6: MT (45 mg/kg) + CA (500 mg/kg) blood samples were collected on day 0, day 6, day 12, day 18, and day 21 from retro-orbital sinus under ether anesthesia and glucose levels were estimated.

Pharmacokinetic study design

Adult albino rats of Wistar strain weighing 180–240 g, the overnight fasted animals were gulped into three groups with six animals each. Group 1 was served as control and received water; Group 2 and Group 3 pretreated with CA extract (500 mg/kg) dissolved in water was administered for 14 days by oral gavage. At the end of the 14th day, MT 90 mg/kg and 45 mg/kg was administered by oral gavage for Group 2 and Group 3, respectively. Blood samples (200 µL each) were collected from tail vein at 0, 0.5, 1, 2, 3, and 4 to 24 h in precoated anticoagulant (K₂EDTA) microcentrifuge tubes. Blood sample were centrifuged at 10,000 rpm for 15 min, plasma sample were separated and were stored in deep freezer (–20°C) for the determination of MT in plasma samples. Protein precipitating technique was employed to separate the drug from plasma and analyzed by reverse phase (RP)-HPLC.

Statistical analysis

PD data obtained were statistically analyzed for variance and significance by One-Way ANOVA followed by Dunnett's multiple comparison test. PK Solver 2.0[®] (Microsoft Inc, USA) validated software was used to measure the PK parameters.

RESULTS

Phytochemical analysis of *Cassia auriculata* L. extract

The analysis for phytochemicals was performed, and the results revealed the presence of alkaloids, carbohydrates, flavonoids, and saponins (21.6%) and showed negative results for sterols. These may be the secondary metabolites involved in anti-hyperglycemic and hypoglycemia activities by involving in the carbohydrate metabolic pathways.

Optimization of chromatographic conditions

Suitable chromatographic conditions are intended into account for the various goals of method development by achieving a good resolution, runtime, sensitivity, and peak symmetry. With the optimized conditions, standard and samples solutions were injected and the chromatograms were recorded. The optimized conditions used for estimation provided a well-defined separation between the drug, internal standard, and endogenous components. The blank plasma samples showed no interference at retention time of the drugs and their internal standards.

The effect of *Cassia auriculata* L. on the pharmacokinetic/pharmacodynamic of metformin

Pharmacodynamic study

A preliminary study was carried out to assess the blood glucose levels of rats followed by 3 weeks treatment with CA in combinations with MT to ensure their potential antidiabetic activity [Figure 1]. The study revealed that CA and MT exhibited the onset of mean glucose lowering effect by day 3 and continuously decreased the fasting glucose levels to normal levels by the end of the study. CA alone treated group had a fair but constant glucose lowering effect when compared with MT, the combination of 90 mg/kg of MT and CA showed enhanced glucose lowering effect of all the groups suggesting the possible synergism and combination of 45 mg/kg of MT and CA had a similar effect of MT alone. Thus from PD data, reduction of dose of MT with CA could be therapeutically significant to MT at 90 mg/kg.

Pharmacokinetic study

The bioanalytical method was developed and validated for MT. Noncompartmental model was employed for evaluating the PK parameter for MT using PK solver 2.0[®] software (Microsoft Inc, USA). Profile of MT at two different doses 90 mg/kg and 45 mg/kg after oral administration in the presence of CA (500 mg/kg) and in the absence of CA was plotted. The PK parameter of MT is shown [Table 1 and Figure 2]. The dose of MT (90 mg/kg) used showed a similar PK profile with that of previously reported study.^[9] The pretreatment of CA produced noticeable changes in the PK parameters of MT. Group II which was pretreated with CA and 90 mg/kg of MT had an increased maximum plasma concentration (C_{max}) by 11.46%. The AUC_{0-24} had a rise of 4.6%. The half-life of the drug nearly reached twice but the time taken to achieve C_{max} (T_{max}) remained the same, in contrast, Group III which had reduced dose to half (45 mg/kg) of MT with pretreated CA had a decreased C_{max} by 17.72%, and AUC_{0-24} 42.42%. T_{max} was doubled with half-life of the drug reaching two-fold when compared with Group I which received only MT.

DISCUSSION

Diabetes mellitus is one of the fastest growing disorders in human kind. The knowledge about the heterogeneity of this disorder increases so as the need of new therapies. There are many allopathic drugs used as a first-line drugs for the treatment of diabetes mellitus, many of them have side effects such as lactic acidosis, severe hypoglycemia, vertigo, and even death.^[10,11] Simultaneously, the use of herbal remedies also gained the equal importance. There is very little clinical evidence to investigate if both are used in combination.

The present work lies in identifying the possibilities of potential interactions of herb and conventional drugs intended for diabetes and

enhancing their therapeutic efficacy when used as combination. As mentioned earlier, the CA is known for its antidiabetic activity,^[12] CA may produce the regeneration of pancreatic β cells which can be clearly evident from the reduction of blood glucose levels to normal in CA (500 mg/kg) treated group suggesting the insulinogenic action and also by improving the impaired glucose hemostasis enhancing the carbohydrate metabolism pathway. PD interactions has been carried out by feeding the standardized plant extracts of CA (500 mg/kg) to streptozotocin-induced diabetic rats along with conventional oral hypoglycemic MT at doses of 45 and 90 mg/kg, respectively. Streptozotocin-induced diabetics provoke various metabolic aberrations such as increased blood glucose, insulin deficiency, and insulin resistance in rats. The results of 3 weeks study demonstrated the antidiabetic potential of CA in combinations with MT to counterbalance the hemostatis related to above parameters comparing the results, a better hypoglycemic control was observed with the combination of higher dose of MT with CA. On 3 weeks treatment, the effect of combination showed superior to the individual treatment and reduction of dose in MT produced a similar glucose lower effect as that of MT alone treated group. As the PK study involves the quantification of standard drugs in the complex biological systems of rat, the bioanalytical method for the quantification of MT from the rat plasma was developed with the slight modification from the previous studies reported.^[9] The developed method had a short run time of 7 min which made the method economic, concise, and precise for analysis of MT in rat plasma. MT is a biguanide class, the first-line drug of choice for the treatment of diabetes mellitus. Absorption half-life of MT when compared with the combination groups has reduced by half indicating the interactions influence the delay of absorption, thus the drug is made available for longer period of time causes the potential interactions and lower dose of MT in combination has closest profile of MT alone which are more evident through PD preclinical data.

The study had some limitations: First, the preliminary study was done in rats; the metabolic pathway and blood glucose regulation are different from humans. Second, PD data were produced to identify the synergism when MT and CA are taken together. Thus, it limited to check the blood glucose levels at regular intervals and further investigations on PD effect

Table 1: Effect of CA on pharmacokinetics of MT

PK parameters	Group 1 (MT-HD)	Group 2 (MT-HD-CA)	Group 3 (MT-LD-CA)
$t_{1/2}$ (h)	7.234±0.5062	14.32±1.187	20.24±7.041
T_{max} (h)	1	1	2
C_{max} (ng/mL)	2545.30±138.8	2837.02±243.1	2094.13±145.9
AUC_{0-t} (ng*h/mL)	17341±849.4	18138±638.4	9984±761.8

PK: Pharmacokinetic; MT: Metformin; AUC: Area under the curve; CA: *Cassia auriculata* L.

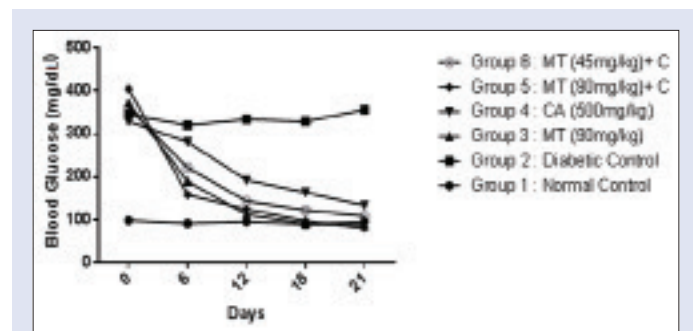


Figure 1: Effect of *Cassia auriculata* L. on blood glucose levels of metformin treated groups

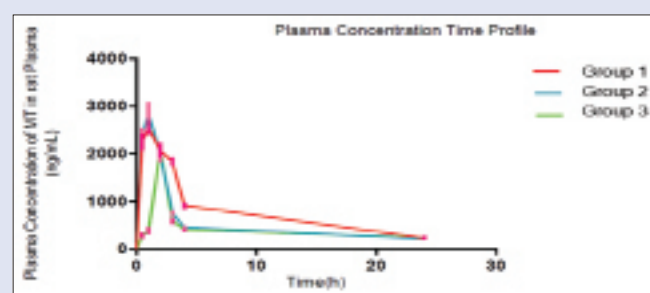


Figure 2: Mean (standard error of mean) plasma concentrations of metformin alone and in combination with *Cassia auriculata* (Pharmacokinetic Profile of Group 1)

may give a clear view in identifying the actual mechanism involved in synergism.

CONCLUSION

Two main problems which travel simultaneously are high dose of MT prescribed, which could cause cumulative dosing, and on the other hand, the risk of herb-drug interactions when co-administrated with phytoconstituents. The present study addressed these problems by elucidating the PK/PD interaction of MT when co-administrated with CA. The results revealed possible potential interaction in PK/PD of MT at higher dose levels, further investigation of reducing the dose level of MT showed no significant change as compared to MT alone at higher dose, thus it is clearly evident to reduce the dose of MT along with CA to achieve the same therapeutic effect of MT alone. However, further study is defensible to apprehend the possible mechanism involved in PK/PD interaction and interpretation of animal data to humans.

Acknowledgment

The authors would like to thank JSS University, Mysore for providing all the facilities to conduct this study and sanctioning the JSS University Research Fellowship Grant to Mr. E. Hemnath (REG/DIR (R)/JSSURF/29 (1) 2010–11) and JSS University Faculty Research Grant to Dr. S. Ponnusankar (REG/DIR (R)/URG/54/2011–12/1845) to conduct this research study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Sivasankaran Ponnusankar obtained his PhD degree in 2011 from Jadavpur University, Kolkata. Currently, he is positioned as Head, Department of Pharmacy practice and has 19 years of teaching experience. He is working on various aspects of herbal pharmacovigilance and herb-drug interaction studies. His area of research expertise is social and administrative pharmacy, pharmaceutical care research, and its outcomes study.

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