EVALUATION OF PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTIONS OF CASSIA AURICULATA WITH METFORMIN IN STREPTOZOTOCIN INDUCED DIABETIC RAT

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ABSTRACT

The present study is an attempt to investigate the pharmacodynamic and pharmacokinetic interactions of Cassia auriculata with Metformin. Though Cassia auriculata’s antihyperglycemic activity has been proved but no work till date has been done to study its Pharmacodynamics and Pharmacokinetic interactions with other oral Hypoglycemics (OH). A single dose of 55 mg/kg streptozotocin prepared in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to overnight fasted animals to induce diabetes. The control rats received an equal volume of citrate buffer and used along with diabetic animals. The One Touch Glucometer AccuChek is used for quantitative in vitro determination of glucose in various samples. Each day after administering drug to the animals after 3 hours a drop of blood was applied on the AccuCheck strip and the blood glucose reading was measured. Diabetes was confirmed after 48 hr of streptozotocin injection, the blood sample were collected through retro-orbital puncture and plasma glucose levels were estimated by one touch Glucometer. The rats having fasting plasma glucose levels more than 250 mg/dL were selected and used for the present study. Cassia auriculata extract was administered for 21 days at single dose level 500 mg/kg made in aqueous and given orally along with the standard drug combinations. The Blood was collected by terminal tail vein puncture and retro orbital puncture. Blood glucose levels were measured using one touch glucometer. Blood was centrifuged at 4000 rpm for 7 minutes. Body weight and blood glucose levels was analyzed every day were analyzed after 21 days. Pharmacokinetically Cassia auriculata and 90 mg/kg Metformin combination increased the $t_{1/2}$, $t_{\text{max}}$ of Metformin, decreased the $C_{\text{max}}$ and AUC$_{0-t}$ was similar to Metformin control. These results suggest that Cassia auriculata and 90 mg/kg Metformin combination is potentiating Metformin’s action by decreasing its elimination, but this may lead to Metformins toxicity. As Cassia auriculata extract itself has antidiabetic activity, it may be a reason why this combination is showing potentiating effect (supra additive). Pharmacokinetically Cassia auriculata and 45 mg/kg Metformin combination is increasing $t_{\text{max}}$, decreasing $t_{1/2}$, increasing $C_{\text{max}}$ and increasing AUC$_{0-t}$ of Metformin.

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INTRODUCTION
The matter of herb-drug interactions appears large over the practice of herbal medicine. Herbal drugs are the plants based products that are used to treat as herbs, herbal supplements, biomedicines or botanicals. When herbal drugs and conventional drugs are used together, they can interact in body, causing changes in the way the herbs and/or the drugs work. Such changes are referred as herb-drug interactions. There are few herb drug interactions reported up till now but they cannot be unobserved as patients who are not aware about the adverse effects that can possibly occur due to concurrent administration of herbal and OTC drugs would have to face devastating consequences. Interactions between herbal drugs depend and prescribed drugs can occur and may lead to serious clinical consequences\(^1\). Herbal therapies can interact with other medications, causing either potentially dangerous side effects and/or reduced benefits from the medication. The nature of herb-drug interactions is not necessarily a chemical interaction between a drug and a herb component to produce a toxic reaction. Instead, the interaction may involve having an herb component cause either an increase or decrease in the amount of drug in the blood stream.

Puranik A.S et al., 2010 suggested that Cassia auriculata technology based seed extract interfered with Metformin’s absorption by decreasing Metformin’s \(C_{\text{max}}\). Even though many such combinations have proved to be beneficial there are several other combinations which are lethal and have not been assessed pharmacodynamically and pharmacokinetically. Cassia auriculata is profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia. It is one of the principle constituents of “Aavaarai panchaga chooranam”- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level\(^2\). Puranik et al., 2010 suggested that Cassia auriculata super critical fluid seed extract interfered with Metformin’s absorption by decreasing Metformin’s \(C_{\text{max}}\). Still no studies on interactions of Cassia auriculata flower with these drugs have been carried out.

MATERIALS AND METHOD:

Plant Extract:
Cassia auriculata flower aqueous extract was procured from M/s Green Chem, Bangalore.

Animals:
Animals were obtained from the central animal house facility JSS College of Pharmacy, Ootacamund, Tamilnadu. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3°C. Animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution. All experiments were performed after obtaining prior approval from IAEC Approval Number JSSCP/IAEC/M.Pharm/Ph.cology/04/2012-2013.

Chemicals and reagent:
Streptozotocin from Aldrich and Sod. Citrate and Acetonitrile from Ramkem, Citric Acid and Phosphoric Acid from Fisher, Sodium Dihydrogen Phosphate from Qualigens, Metformin and Glimepiride from Cipla, Mumbai. All other chemicals used in the studies were analytical laboratory grades procured from the following manufacturers, Merck Lab, S.D Fine chemicals.

PHARMACOLOGICAL STUDIES

Induction of diabetes
A single dose of 55 mg/kg streptozotocin prepared in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to overnight fasted animals to induce diabetes. The control rats received an equal volume of citrate buffer and used along with diabetic animals.

Assessment of Diabetes
Diabetes was confirmed after 48 hr of streptozotocin injection, the blood sample were collected through retro-orbital puncture and plasma glucose levels were estimated by one touch Glucometer. The rats having fasting plasma glucose levels more than 250 mg/dL were selected and used for the present study.

One Touch Glucometer AccuCheck Method: The method is used for quantitative in vitro determination of glucose in various samples. Each day after administering drug to the animals after 3 hours a drop of blood was applied on the AccuCheck strip and the blood glucose reading was measured.

Experimental Design:
The Wistar rats weighing 180-250 gm of either sex were used for the experimental study. The animals were divided into six groups of 6 animals in each for each pharmacokinetic and pharmacodynamics studies.
Table 1: Animal Grouping for Pharmacodynamics and Pharmacokinetic Study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Metformin (90 mg/kg)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Metformin (90 mg/kg) + <em>Cassia auriculata</em> aqueous flower extract (500 mg/kg)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Metformin (45 mg/kg) + <em>Cassia auriculata</em> aqueous flower extract (500 mg/kg)</td>
</tr>
</tbody>
</table>

Pharmacodynamics studies:
*Cassia auriculata* extract was administered for 21 days at single dose level 500 mg/kg made in aqueous and given orally along with the standard drug combinations. The blood was collected by terminal tail vein puncture and retro orbital puncture. Blood glucose levels were measured using one touch glucometer. Blood was centrifuged at 4000 rpm for 7 minutes. Body weight and blood glucose levels was analyzed every day were analyzed after 21 days.

Pharmacokinetics studies:
*Cassia auriculata* extract was administered for 14 days at single dose level of 500 mg/kg made in aqueous and given orally. On the 14th day standard drugs were given along with herbal extract. Blood was collected through retro orbital puncture under light diethyl ether anesthesia. The blood was collected at following intervals: 0, ½, 1, 2, 3, 4, & 24th hour. The blood was centrifuged at 4000 rpm for 7 minutes. Plasma was separated and analyzed using HPLC technique.

Parameters observed:

Antidiabetic activity
- Plasma glucose estimation (initial and final)
- Body weight of the animals (initial and final)

Determination of drug concentration in plasma using HPLC.

Blood sampling and plasma separation:
Un-haemolysed sample of blood was collected from the clean tail tips/retro orbital in eppendroff tubes from the anaesthetized animals. The blood was centrifuged at 4000 rpm for 7 minutes to separate the plasma and subjected to Quantitative estimation using HPLC (High Performance Liquid Chromatography).

RESULTS AND DISCUSSION
Pharmacodynamics results:
The effect of aqueous extract of flowers of *Cassia auriculata* at 500 mg/kg on glucose levels using streptozotocin induced diabetic model in rats was studied using Metformin at two dose levels (90 mg/kg & 45 mg/kg) and Glimepiride at two dose levels (0.54 mg/kg & 0.27 mg/kg). The results were recorded in Table 2 & Table 3.

Table 2: Blood Glucose measurements at intervals 0, 7, 14 and 21 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp I [Met H.D (90 mg/kg)]</td>
<td>411 ± 8.136</td>
<td>305 ± 7.718</td>
<td>333 ± 11.644</td>
<td>283 ± 16.793</td>
</tr>
<tr>
<td>Grp II [Met H.D (90 mg/kg) + Extract (500 mg/kg)]</td>
<td>405 ± 5.665</td>
<td>287 ± 9.807</td>
<td>329 ± 30.586</td>
<td>224 ± 7.714</td>
</tr>
<tr>
<td>Grp III [Met L.D (45 mg/kg) + Extract (500 mg/kg)]</td>
<td>434 ± 10.960</td>
<td>552 ± 6.349</td>
<td>495 ± 7.242</td>
<td>386 ± 2.513</td>
</tr>
</tbody>
</table>

Table 3: Blood Glucose measurements at intervals 0, 7, 14 and 21 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Reduction in Blood Glucose Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Metformin 90 mg.kg)</td>
<td>31 %</td>
</tr>
<tr>
<td>Group 2 (Metformin 90 mg.kg) + Extract (500 mg/kg)</td>
<td>45 %</td>
</tr>
<tr>
<td>Group 3 (Metformin 45 mg.kg) + Extract (500 mg/kg)</td>
<td>11 %</td>
</tr>
</tbody>
</table>
Effect of Cassia auriculata flower aqueous extract on 90 mg/kg Metformin:

Cassia auriculata and 90 mg/kg of Metformin combination did not significantly change blood glucose level when compared with Metformin alone (90 mg/kg) and Cassia Auriculata-Metformin High Dose (Group II) combination treated groups. (p>0.05) (Table 2 & Fig. 1)

Effect of Cassia auriculata flower aqueous extract on 45 mg/kg Metformin:

Cassia auriculata and low dose of Metformin combination significantly changed blood glucose level when compared with Metformin alone (Group I) and Cassia Auriculata-Metformin low Dose (Group III) combination treated groups. (p<0.05) (Table 2 & Fig. 1)

Effect of Metformin:

Metformin when given alone at a single dose level (90 mg/kg) to Group I animals showed significant reduction in the blood glucose levels on 0th day, 7th day, 14th day and 21st days also the mean body weights of the animals were also maintained. (Table 2 & Fig. 1)

![Blood glucose levels of when metformin given in combination with C. auriculata and alone](image)

**Fig. 1:** Blood glucose levels of when metformin given in combination with *C. auriculata* and alone

Values are mean ± SEM; n=6 in each group. *P>0.05, *P<0.05 when compared with Metformin Control; One-way ANOVA followed by Dunnets multiple comparison test.

**Pharmacokinetic results:**

The effect of aqueous extract of flowers of cassia auriculata at 500 mg/kg on pharmacokinetic parameters (t₁/₂, Tₘₐₓ, Cₘₐₓ, AUC₀₋ₜ) was studied using Metformin at two dose levels (90 mg/kg & 45 mg/kg) and Glimepiride at two dose levels (0.54 mg/kg & 0.27 mg/kg). The results were recorded in Table 7 & 8.

Effect of Cassia auriculata flower aqueous extract on 90 mg/kg Metformin:

Cassia Auriculata aqueous extracts at a dose of 500mg/kg when co-administered with High Dose of Metformin (90 mg/kg) increased the AUC₀₋ₜ by 2% than Metformin alone at dose (90 mg/kg), decreased the Cₘₐₓ by 17%, increased the tₘₐₓ by 50%, and increased the t₁/₂ by 71% of Metformin when given in combination. (Table 7 & Fig. 8)

Effect of Cassia auriculata flower aqueous extract on 45 mg/kg Metformin:

Cassia Auriculata aqueous extracts at a dose of 500mg/kg when co-administered with Low Dose of Metformin (45 mg/kg) increased the AUC₀₋ₜ and Cₘₐₓ by 2% and 10% than the Metformin alone at dose (90 mg/kg), tₘₐₓ was same and the t₁/₂ was reduced by 16%. (Table 7 & Fig. 8)
Effect of Metformin:
When Metformin was given in a single dose of (90 gm/kg) the AUC 0-t was found to be 94.3182802 μg/ml*h, the C$_{\text{max}}$ was 25.45298014 μg/ml, the $t_{\text{max}}$ was 1 hr, and $t_{1/2}$ was 4.988481722 hrs. (Table 4 & Fig. 2)

Table 4: Pharmacokinetics of Metformin and Cassia auriculata

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>Units</th>
<th>GROUP I Metformin (90 mg/kg)</th>
<th>GROUP II Metformin (90 mg/kg) + Extract (500 mg/kg)</th>
<th>GROUP III Metformin (45 mg/kg) + Extract (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t$_{1/2}$</td>
<td>h</td>
<td>4.988481722</td>
<td>17.43919356</td>
<td>4.185984019</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>h</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C$_{\text{max}}$</td>
<td>μg/ml</td>
<td>25.45298014</td>
<td>20.94252216</td>
<td>28.37033818</td>
</tr>
<tr>
<td>AUC 0-t</td>
<td>μg/ml*h</td>
<td>94.3182802</td>
<td>96.02456781</td>
<td>96.18133779</td>
</tr>
</tbody>
</table>

Both CA and Metformin are anti-hyperglycemic agents and therefore the blood glucose in control and experimental groups may not have significantly changed. The CA-HA extract was well tolerated without any adverse events in both male and female rats. The pharmacokinetic herb–drug interaction is an important aspect which needs be included in safety pharmacology studies. It is predicated that herb–drug interactions may be under-reported, under-estimated and probably may occur more frequently than the drug–drug interaction. The CA-HA extract when co-administered with Metformin (500 mg/kg) did not interfere with pharmacokinetics. However, co-administration with CA-SFE extract at 250 (P<0.05), 500 (P<0.01) and 1000 mg kg$^{-1}$ (P<0.05) decreased Metformin concentration, at the earliest time point of 15 min. This trend continues in CA-SFE 500 and 1000 mg kg$^{-1}$. In CA-SFE 500 mg kg$^{-1}$ the C$_{\text{max}}$ of Metformin is delayed by 1.5 h; however; in CA-SFE 1000 mg kg$^{-1}$ there is a significant (P<0.01) decrease in Metformin’s C$_{\text{max}}$ indicating inhibition in Metformin’s absorption. This clearly suggests that the CA-SFE interferes with the absorption of Metformin which can be explained by the presence of non-polar components. Metformin has negligible plasma protein binding and it undergoes active renal tubular excretion by organic cation transporter (OCT2). Modulation of OCT2 would have changed the elimination pattern; however, we did not observe any such changes when co-administered with Metformin. Hence, it is recommended that co-administration of CA-SFE with modern medicine should be avoided unless supporting evidence base especially on safety and pharmacokinetics is available$^3$.

Mechanism by which CFET brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with CFET. Increase in the level of haemoglobin in animals given CFET may be due to decreased level of blood glucose and glycosylated haemoglobin. CFET administration to streptozotocin dosed animals reversed the weight loss. The ability of CFET to recover body weight loss seems to be due to its antihyperglycemic effect$^{4,5}$.  

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Hydromethanolic, n-butanol and ethyl acetate fraction shown significant ($P < 0.001$) reduction in blood glucose level. However n-butanol fraction was highly effective and results are comparable with that of reference drug, Phenformin. Alloxan treated rats shown substantial weight loss and also affect carbohydrate, lipid and protein metabolism. All three extracts were found to be effective in restoring the body weight of animals to the normal value. Elevated blood lipids especially cholesterol and triglycerides as well as reduction of protein level are other indicators of diabetic condition. Diabetic animals treated with hydromethanolic extract as well as ethyl acetate and n-butanol fraction shown significant ($P<0.001$) effect on serum protein, cholesterol and triglyceride level. In comparison to other extracts, n-butanol fraction was found to be more potent in normalizing the blood lipids and protein level. Proximate chemical analysis reveals the presence of steroids, flavonoids, tannins and carbohydrates in hydromethanolic extract where as ethyl acetate and n-butanol fractions were found to be very rich in flavonoid content. Thus, flavonoids from *Cassia auriculata* Linn. May be responsible for its antidiabetic potency.\(^6\)

Uma Devi et al, 2006 observed that aqueous extracts of flower and leaves of *Cassia auriculata* decreases blood glucose in alloxan diabetic rats. The reason may be flowers and leaves contain more constituents such as alkaloids, steroids and tannins. As per literature, alkaloids, steroids and tannins are known to reduce blood glucose level in diabetic condition. The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycemic Sulphonylureas, Tolbutamide, that promote insulin secretion by closure of K⁺-ATP channels, membrane depolarization and stimulation of Ca²⁺ influx, an initial key step in insulin secretion\(^7,8\).

**CONCLUSION**

Statistically *Cassia auriculata* and 90 mg/kg Metformin combination did not significantly reduce the blood glucose levels when compared to Metformin control (Fig. 1). Pharmacokinetically *Cassia auriculata* and 90 mg/kg Metformin combination increased the \(t_{1/2}\), \(t_{max}\) of Metformin, decreased the \(C_{max}\) and \(AUC_{0,t}\) was similar to Metformin control (Table 4). These results suggest that *Cassia auriculata* and 90 mg/kg Metformin combination is potentiating Metformin’s action by decreasing its elimination, but this may lead to Metformins toxicity. As *Cassia auriculata* extract itself has antidiabetic activity, it may be a reason why this combination is showing potentiating effect (supra additive). From Fig. 2, it can be inferred that AUC of Metformin when administered in combination with *Cassia auriculata* extract was reduced when compared to Metformin alone. Cassia auriculata and 45 mg/kg Metformin combination has significantly reduced the blood glucose levels as compared to Metformin control (Fig. 6). Pharmacokinetically Cassia auriculata and 45 mg/kg Metformin combination is increasing \(t_{max}\), decreasing \(t_{1/2}\), increasing \(C_{max}\) and increasing \(AUC_{0,t}\) of Metformin (Table 4). These results indicate that cassia auriculata and 45 mg/kg Metformin combination is showing additive synergism. As the dose of Metformin is less, so their combination will show less/no toxicity as compared to Cassia auriculata with 90 mg/kg of Metformin.
REFERENCES