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PHARMACOKINETIC AND PHARMACODYNAMIC DRUG INTERACTION OF METFORMIN AND HERBAL EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: The synthetic and herbal drugs are commonly used by the patients in chronic diseases like diabetes mellitus, cancer and hepatitis. **Objective:** The present study was designed to investigate the possible drug- drug interactions between the methanolic extract of *Momordica dioica* seeds and Metformin. **Materials and Methods:** Male Wistar rats weighing about 180-250 gms were selected for the study. The type II diabetes was induced in animals by a single intra peritoneal injection of streptozotocin (30 mg/kg body weight). The animals were divided into 8 groups of 6 rats each (n=6). Metformin was administered orally as – 20, 40 and 80 mg/kg, and metformin in combination with herbal extracts were administered in same doses. The effect of combination of both drugs on the pharmacokinetic parameters was studied in experimental animals after the treatment for a period of 21 days. The pharmacodynamic effect of combination of both the drugs was studied on the serum glucose levels of diabetic rats after multiple dosing. **Results:** It is evident from the study that the serum glucose levels with metformin in the dose of 40 mg/kg along with the herbal extract was significantly decreased ($^{*}p<0.05$). The results proved that pharmacokinetic and pharmacodynamic interactions between the Metformin and *Momordica dioica extract* are significant. **Conclusion:** The study concludes that the dose of metformin, can be reduced in combination with herbal drug, to minimize the adverse effects in long term use in diabetes. The study undertaken has clinical significance of such drug combinations in therapy.

KEYWORDS: Streptozotocin, diabetes, Pharmacodynamic interaction, Metformin, Area under curve.

INTRODUCTION

Herbal drugs play an important role in today's human life. In developing countries, people rely more on herbal drugs and this trend is growing, due to high prices and potential side effects of synthetic drugs. The reason might be because of devoid of toxic effects, less price and good patient compliance.[1] In developing countries, 80% of people utilize plants to meet their primary health care needs. Approximately 25% of today's prescription contains drugs from plant extracts. There are millions of people who use herbs either as food or in the form of medicine along with prescription and non-prescription medications. According to WHO, by the year 2050, the number of medicinal plants would be reaching nearly US $ 5 trillion. They can be administered in combination with therapeutic drugs, raising the potential of herb-drug interactions. When herbal medications are given in combination with other drugs, they either produce side effects or potentiating the action of other medication. According to few reports, the drug interactions may be fourth to sixth leading cause of death globally.[2]

A major concern for the health care professionals and their patients is a metabolic drug interaction between drugs. The incidence of drug-drug interaction ranges from 3-5% who takes few drugs and 20 % in patients receiving 10-20 drugs. Hence, in clinical practice it is necessary to understand and establish such interactions. The underlying mechanisms in drug interactions can be better studied in animal models, as in human models, clinical observations are critical to note the drug interactions.[3]

Mechanisms of herb-drug interactions:

The mechanisms of drug interactions can be divided into two categories:

1) Pharmacokinetic interactions which influence absorption, distribution, metabolism or excretion of a drug (ADME) and thus lead to increased or reduced plasma levels of a drug; and
2) Pharmacodynamic interactions, which cause changes in pharmacological responses of the drug through additive, synergistic or antagonistic actions. Unfavorable effects may incur, causing target toxicity, if the effect of the drug in combination with the herbal medicine is enhanced synergistically or by additive effects.[4]

Momordica dioica Roxb. Ex. Wild is a perennial, dioceous climbing creeper belonging to family Cucurbitaceae. Its common name is Parora, kakora. Flowering occurs during June to July and fruiting during September to November. This is climbing creeper generally found throughout India, Pakistan, Bangladesh, Himalayas to Ceylon. Kakrol is a Cucurbitaceous crop originated in the Indo –Malayan region.[5] Fruits of the plant are green and generally used as vegetable. It possesses many medicinal properties like diuretic, alexiteric, stomachic, laxative, hepatoprotective, and has antivenum property. It is also used to cure asthma, leprosy, excessive salivation, prevent the inflammation caused by lizard, snake bite, elephantiasis, fever, mental disorders, digestive disorders and troubles of heart and to treat discharge from mucous membrane.[6] Fresh fruit juice is prescribed for hypertension. Tender fruits are rubbed on skin for pimples and acne. Seeds are roasted and taken for eczema and other skin problems. Leaves of the plant are anti-helminthic, aphrodisiac. They are also used to cure tridosha, fever and alter pitta, jaundice, asthma, bronchitis, piles, hepatic damages, mental digestive disorders, bleeding piles bowel affection and urinary complaints. The juice of the leaves mixed with coconut, pepper, red sandalwood etc in order to form an ointment and applied to the head to relieve pain in the head. Roots of the *Momordica dioica* are full of medicinal values.[7] Literature review reveals that the *M. dioica* leaves produce hepatoprotective activity of extract on CCl_4 induced hepatic oxidative stress. CCl_4 intoxication displayed elevated level of serum AST, ALT, ALP and total bilirubin. Thus, the study reveals that possible mechanism of this activity may be due to free radical-scavenging and antioxidant activities which may be due to the presence of flavonoids in the extracts.[8] A study also proved that seed extract of *M. dioica* also possess anti-hyperglyceamic action which could be possible due to a decrease in insulin resistance or improvement in the sensitivity of insulin.[9]

Diabetes mellitus is a metabolic disorder associated with high blood glucose levels, either due to deficiency in production of insulin by the pancreas or due to insulin resistance. There are two types of diabetes: Type I diabetes mellitus is also called as insulin dependent diabetes mellitus (IDDM) which is produced mainly due to mere production of insulin and Type II diabetes mellitus as non-insulin-dependent diabetes mellitus (NIDDM) which is produced mainly due to decreased sensitivity of insulin.[10]

Metformin belongs to the class biguanide and is oral anti-hyperglycemic agent used as a single therapy or in combination with other therapies for the treatment of type 2 diabetes. It

acts by reducing the insulin resistance and hepatic glucose production, improves insulin sensitivity by elevating the peripheral glucose uptake and utilization. However, in humans the bioavailability of metformin is dose-dependent with absorption which occurs in gastrointestinal tract and small intestine. The adverse effects of the drug are unusual tiredness, dizziness, severe drowsiness, chills, blue/cold skin, muscle pain, fast/difficult breathing, slow/irregular heartbeat, stomach pain with nausea, vomiting, or diarrhea.[11]

The present study was intended for studying pharmacodynamic and pharmacokinetic interactions between metformin and *Momordica dioica* in rats.

MATERIALS AND METHODS

Collection of Plant material and extraction

Fresh fruits of *M. dioica* (5 kg) were collected locally, during July-August, 2011 and seeds were removed mechanically and dried under shade. They were identified and authenticated by Sri Venkateshwara University, Botany department, Tirupati. The seeds were powdered in electric grinder. The powder was subjected to methanol extraction, in Soxhlet apparatus and was run about 10 cycles. After filtration through Whatman filter paper, the filtrates were dried in desiccator. The methanolic extract is dissolved in Tween 80 and administered orally at the dose of 200 mg/kg body weight and the synthetic drug Metformin Hcl (Hetero drugs Pvt Ltd) is dissolved in distilled water and administered orally at different doses of 20, 40 and 80 mg/kg body weight.

Preliminary Phytochemical analysis

Preliminary Phytochemical analysis was carried out for the presence of alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids and saponins in methanol extract of *M.dioica*.[12]

Animals

Animal Protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experimentation on Animals) through its reference no: IAEC/SVCP/2016/001, Dated: 27/02/2016. Male Albino wistar rats, weighing (180-250 gms) were obtained from NIN (National Institute of Nutrition, Hyderabad. The animals were acclimatized to the experimental room at a temperature of 23 ± 2^0 C, controlled humidity conditions (50-55%) and 12 hr light and 12 hr dark cycles.

They were fed with standard food pellets (Hindustan Lever, Hyderabad) and water *ad libitum*.

The animals are divided into 8 groups of 6 animals each.

Group I: Normal

Group II: Diabetic control (Rats which develop > 250 mg/dl)

Group III: DC + Metformin low dose (20mg/kg)

Group IV: DC + Metformin moderate dose (40 mg/kg)

Group V: DC + Metformin high dose (80mg/kg)

Group VI: DC + Methanolic extract of *M.dioica* (200 mg/kg) + Metformin low dose (20mg/kg)

Group VII: DC + Methanolic extract of *M.dioica* (200 mg/kg) + Metformin moderate dose (40mg/kg)

Group VIII: DC + Methanolic extract of *M.dioica* (200 mg/kg) + Metformin high dose (80 mg/kg)

Acute toxicity studies

Methanol extracts of *M.dioica* seeds were studied for acute oral toxicity as per OECD (Organization for Economic Cooperation and development, 2001) guidelines No. 423 (2000). The extract did not produce any signs of toxicity when given in doses up to 2000 mg/kg by an oral route. Hence, a dose of 200 mg/kg dose of the extract was selected.[13]

Induction of Diabetes[14]

Induction of Experimental Diabetes: The animals were fasted for 12 h prior to the induction of diabetes. Diabetes was induced by a single intraperitoneal injection (i.p) of a freshly prepared Streptozotocin (STZ) solution (Dose: 30-60mg/kg) in acetate buffer 0.1 M, pH 4.5 to rats. Control rats were received only the buffer. Diabetes was identified by polydipsia, polyuria and by measuring non-fasting blood glucose levels 48 h after injection of STZ. Rats with blood glucose levels of above 250 mg/dl were considered to be diabetic and used for the studies. MEMD (Methanolic extract of *M.dioica*, 200 mg/kg) and Metformin at doses of 20, 40 and 80 mg/kg) were administered to diabetic rats for a period of 21 days and the serum glucose and pharmacokinetic parameters such as Cmax, tmax and AUC were determined.[14]

Preparation of Standard solutions

Accurately weighed quantity of synthetic drug (100mg) is dissolved in HPLC grade Acetonitrile:Water (45:55% v/v) and volume is made up to 100ml in volumetric flask (Stock A). From this stock solution 1 ml of solution is taken in 100 ml volumetric flask and the volume is made up Acetonitrile:Water (Stock B). Different dilutions are prepared in the concentration range of 20,40,60,80,100 and 120 µg/ml. Calibration standards are prepared by spiking the drug from the serially diluted solutions into the blank serum and absorbance is measured at 230 nm.[15]

Preparation of sample preparation was done in the same way as standard.

Collection of blood samples and preparation (procedure)

The blood will be collected from the retro-orbital plexus in the presence of mild ether. The collected blood samples are centrifuged at 2000 rpm for 20 min at 25°C and plasma is separated. The centrifuged plasma samples will be stored at -20°C until analyzed. To aliquot of 500 µl of plasma samples, 1.5 ml of Mobile phase is added and vortex mixed for 1 min to ensure complete precipitation and centrifuged at 10,000 rpm at 4 °C for 20 min. Supernatant of the centrifuged samples are collected and analyzed using HPLC.

HPLC assay for Synthetic drug

The quantitative determination of drug in plasma was performed by HPLC assay using following chromatographic conditions.

Chromatographic conditions

Mobile phase: Acetonitrile: Water (45:55% v/v)

Stationary phase: Phenomenex Gemini C18 (4.6 ×150 mm, 5µ)

Flow rate: 1.0 ml/min

Runtime: 10 minutes

Injection volume: 20µl

Temperature: 30˚C

Wavelength: 230 nm

Internal standard: Glipizide (40µg/ml)

Pharmacokinetic analysis (Table 3) was done. Plasma concentration – time curve was plotted, and the peak plasma concentration (Cmax) and time needed to reach the peak plasma

concentration (Tmax) were noted directly from the generated data. The area under the plasma level-time curve ($AUC_{0-\infty}$) was calculated using Trapezoidal rule. The elimination rate constant (K_{cl}) was calculated from the semi-log plot of the data using slope of the terminal elimination phase; and half-life ($t_{1/2}$) was calculated by 0.693/ K_{cl}.[16]

Statistical analysis

Results were expressed as the mean ± S.E.M. for Statistical analysis of the data group, means was compared by one-way analysis of variance (ANOVA) followed by Dunnette's test. $p<0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical analysis

Preliminary Phytochemical analysis showed the presence of alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids and saponins in methanol extract of *M.dioica*.

Effect of MEMD on blood glucose levels

MEMD (200mg/kg) and metformin (50 mg/kg) showed significant ($p<0.001$) fall in blood glucose levels at 1hr, 2 hr and 4 hr respectively when compared with diabetic control group (Table 2).

Table 1. Effect of MEMD and Metformin on blood glucose level in diabetic rats.

Groups	Day 0	Day 8	Day 15	Day 21
Group – I	94.4 ± 3.80	86.5 ± 3.21	91.3 ± 2.03	85 ± 0.36
Group – II	315 ± 2.62[a]	300 ±1.96[a]	306 ± 2.69[a]	296 ± 2.50[a]
Group – III	300 ± 1.20	295 ± 1.40	272 ± 2.02	255 ± 2.62
Group – IV	315 ± 2.02	289 ± 1.09[a]	260 ± 2.63[b]	273 ± 3.12[b]
Group - V	286 ± 1.02[a]	250 ± 1.69[a]	220 ± 3.21[b]	119 ± 2.79[b]
Group - VI	310 ± 1.69	295 ± 1.12	270 ± 1.86	250 ± 2.69
Group - VII	295 ± 1.69	266 ± 1.70	176 ± 1.97[b]	125 ± 1.76[b]
Group - VIII	300 ± 2.76	280 ± 3.12	239 ± 1.90[b]	163 ± 1.80[b]

The data are expressed in Mean±SEM. n=6 in each group.

[a]p [b]p [c]p compared with corresponding value of diabetic animals.

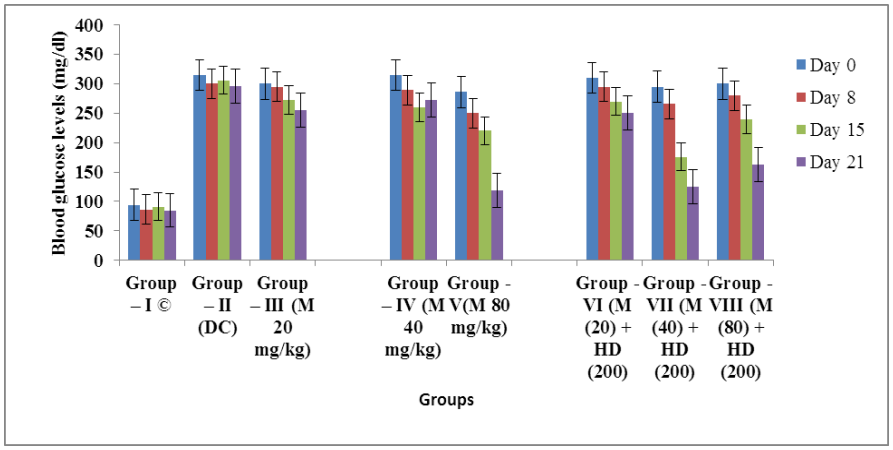


Figure 1: blood glucose levels in mg/dl in different groups M – Metformin, HD – herbal drug.

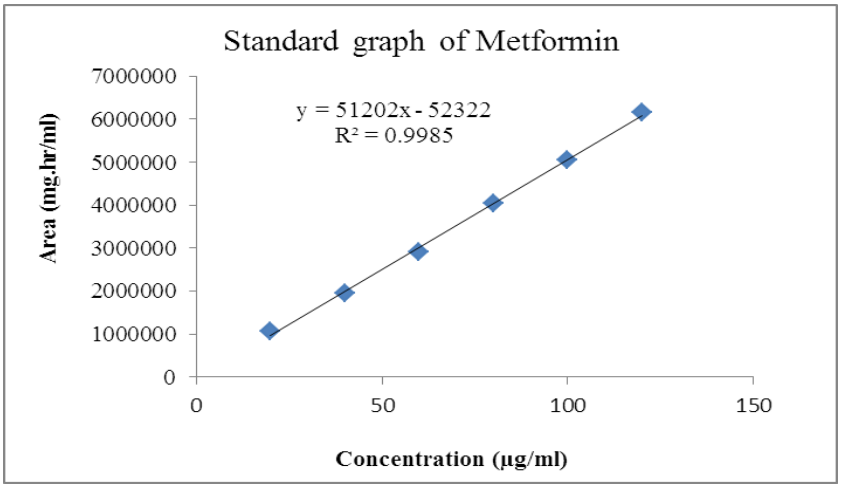
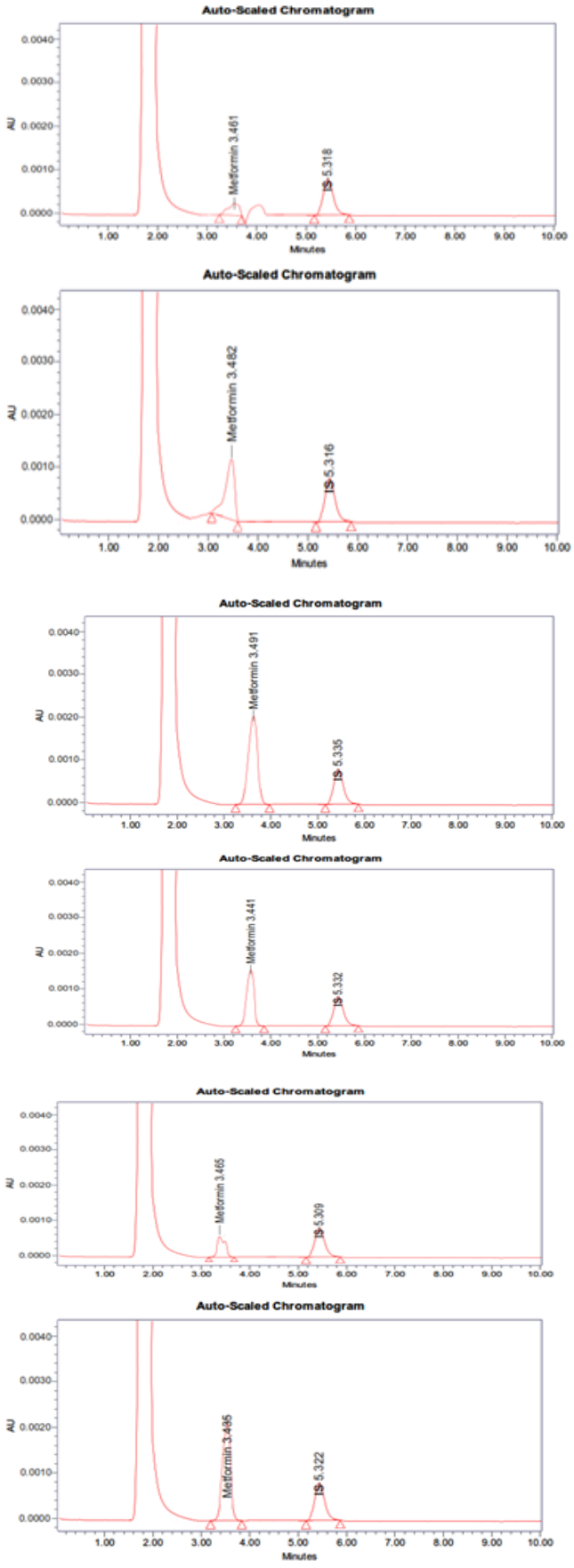


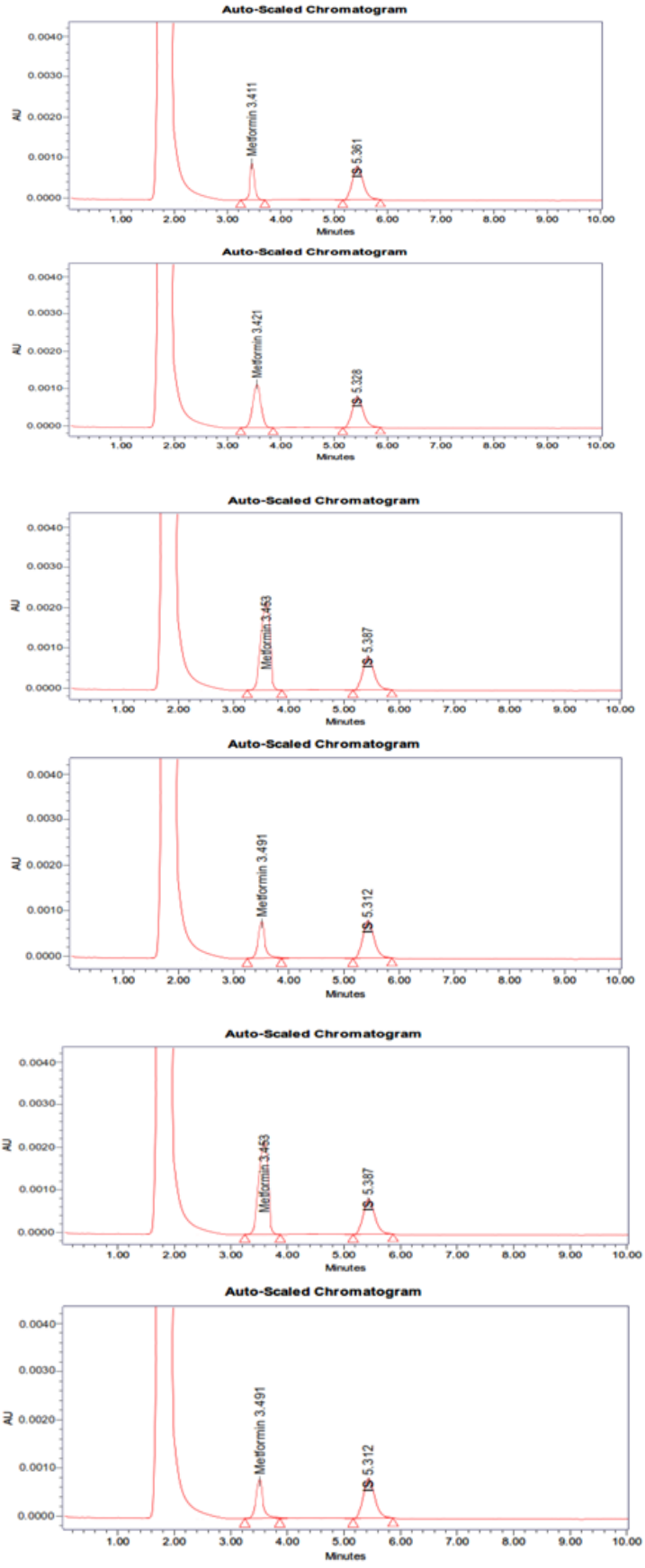
Figure 2: Standard graph of Metformin.

Table 2. Concentration of metformin with area.

Concentration (µg/ml)	Area (mg.hr/ml)
20	1076185
40	1944757
60	2919086
80	4045333
100	5048604
120	6156889



Chromatograms of different groups



Chromatograms of different groups

Table 3. Mean value of pharmacokinetic parameters of metformin in treated groups.

Groups	$(AUC)_{(0-\infty)}$	T_{max} (hrs)	c_{max}	$t_{1/2}$
Group – III (Metformin-20 mg/kg)	1.787±0.01	4±0.2	1.13±0.12	173.25±0.2
Group – IV (Metformin-40 mg/kg)	1.687±0.02	4±0.5	1.18±0.01	43.3±0.4[a]
Group – V (Metformin-80 mg/kg)	2.793±0.01[a]	4±0.9	1.33±0.02	16.11±0.9[a]
Group – VI (Metformin-20 mg/kg+*M.dioica* (200mg/kg)	1.638±0.03	4±0.7	1.15±0.01	13.86±0.4[a]
Group – VII (Metformin-40 mg/kg+*M.dioica* (200mg/kg)	2.870±0.02[c]	4±0.3	1.34±0.01[c]	11.17±0.9[a]
Group – VIII (Metformin-80 mg/kg+*M.dioica* (200mg/kg)	1.361±0.01	4±0.8	1.18±0.02	25.66±0.8[a]

The data is expressed in Mean±SEM. n=6 in each group.

[a]p [b]p [c]p compared with corresponding value of diabetic animals.

DISCUSSION

In 21st century, diabetes is considered as one of the most challenging health problems. Persistent high glucose levels cause many complications such as diabetic neuropathy, diabetic nephropathy, stroke and diabetic retinopathy. Type 2 diabetes mellitus is treated with monotherapy or combination therapy. The uncontrolled blood glucose levels in elderly patients may depend on multiple-targeted treatments which include, anti-hypertensive drugs, anti-hyperlipidemics, anti-platelet agents to combat with the complications of diabetes mellitus. In this regard, other than the routinely prescribed drugs, diabetic patients after consume herbal preparations to control the glucose levels in blood. The co-administered herbs may potentiate/antagonize pharmacological effects of leading to some effects. Keeping in view about the growing complications of choices for the treatment of type - 2 diabetes, it is important to ponder drug-drug and drug-herb interactions between various anti-hyperglycemic agents.

The main objective of the study was to evaluate the effect of *Momordica dioica* on pharmacokinetic and pharmacodynamics of metformin in diabetic rats. Diabetes was induced by using streptozotocin. Streptozotocin (STZ) is most commonly used to induce diabetes in rats. This causes the death of pancreatic β-cell by alkylation of DNA resulting in reduced synthesis and release of insulin. This results in fragmentation of DNA by means of

production of reactive oxygen species. STZ selectively destroys the pancreatic cells that secrete insulin, which causes less active pancreatic cells and produces diabetes mellitus. The STZ induced diabetic rats showed significant increase in blood glucose levels (17). STZ induction is associated with formation and accumulation of free radicals which leads to a number of deleterious effects. The blood glucose reduction was high in combination of *M.dioica* and metformin (40 mg/kg) as compared to metformin group alone at the dose of 40 mg/kg. However, at higher dose of metformin (80 mg/kg), metformin showed a significant reduction in blood glucose levels alone than in combination with herbal drug. The glycemic control of herbal drug may be due to the presence of phytochemical constituents like alkaloids, flavonoids, saponins and tannins. There can be changes in pharmacokinetic parameters of metformin with the concurrent administration of herbal drug *M. dioica*. Combination therapy has shown significant ($p<0.05$) increase in Cmax, AUC of metformin as compared to metformin alone group. Increased values of $AUC_{(0-\infty)}$ indicated increased bioavailabilty of metformin in the presence of *M.dioica*, and the effect was more in combination of *M.dioica* at the dose of (40 mg/kg) treated group as compared to other groups. The Cmax was found to be 1.34 µg/ml at Tmax of 4 hrs in combination of herbal drug with metformin (40 mg/kg) and $t_{1/2}$ of 11.17 hrs as compared to the other groups. The short $t_{1/2}$ indicates that the drug metformin will not be retained, and gets eliminated faster as compared to the other groups.

Any conventional drug like metformin, glimipiride, glipizide or any other oral hypoglycemic agent produces adverse effects on use of long-term. Hence, it is always advisable to reduce the dose and administer the drug in order to minimize the adverse effects. This can be possible only when the dose of conventional drug is reduced in combination with herbal drug.

CONCLUSION

The results of the above study proved that pharmacokinetic and pharmacodynamic interactions were found between metformin and *Momordica dioica. M.dioica* is altering the pharmacokinetic parameters of Metformin in rats by significantly increasing Cmax, tmax and $AUC_{(0-\infty)}$. The combination of metformin (40mg/kg) with M.dioica (200 mg/kg) had shown significant reduction in blood glucose levels at 15^{th} and 21^{st} day respectively as comparable to the other groups. Hence, attention in doses is required if used along with *M. dioica* to avoid the adverse effects and also complications due to increased bioavailability of metformin.

However, extensive clinical pharmacokinetic studies are necessary to establish drug-herbal interactions.

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