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A PKPD interaction between *Momordica charantia* and oral hypoglycemic drug – dapagliflozin in STZ induced hyperglycemic rats

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Abstract

Traditional medications obtained from the medicated herbal plants are used by about maximum percentage of world population for different chronic disease condition. Diabetes (Hyperglycemia- high blood sugar level) is a very important metabolic disorder in different developed, developing countries including India. It providing to very serious complications on health of human beings, especially in the rural and subruralareas. Hence, the research studies required to be subjected to pharmacodynamic and pharmacokinetic studies in order to determine effect of *Momordica charantia* herb on the hyperglycaemic patients who are taking the therapy with synthetic drugs. This study was to discover the influence of *Momordica charantia on* the pharmacokinetics and pharmacodynamics of Dapagliflozinand in rats**.** Results have proved the negative (decrease) effect of *Momordica charantia on* pharmacokinetics but positive (increase) effect on pharmacodynamics of Dapagliflozin.

Keywords: *Momordica cherantia*; Dapagliflozin Pharmacokinetics; Pharmacodynamic

1. Introduction

Diabetic Mellitus (Hyperglycemia) is an endocrine disease and not a single disease which is a group of chronic metabolic orheterogeneous affliction due to the irregular secretions of insulin and action of insulin or both. Absence orreduced insulin in turn leads to abnormal high blood sugar level and glucose intolerance [1-5].

Dapagliflozin is available as a film-coated tablet for oral administration containing the equivalent of 5 mg dapagliflozin as dapagliflozinpropanediol or the equivalent of 10 mg dapagliflozin as dapagliflozinpropanediol, and the following inactive ingredients: microcrystalline cellulose, anhydrous lactose, crospovidone, silicon dioxide, and magnesium stearate. In addition, the film coating contains the following inactive ingredients: polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, and yellow iron oxide. Sodium-glucose cotransporter 2 (SGLT2), expressed in the proximal renal tubules, is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. Dapagliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, dapagliflozin reduces reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion. *Hypotension:* Before initiating FARXIGA, assess volume status and correct hypovolemia in the elderly, in patients with renal impairment or low systolic blood pressure, and in patients on diuretics. Monitor for signs and symptoms during therapy.

Monitor renal function during therapy.*Hypoglycemia:* In patients taking insulin or an insulin secretagogue with FARXIGA, consider a lower dose of insulin or the insulin secretagogue to reduce the risk of hypoglycemia.*Genitalmycotic infections:* Monitor and treat if indicated. *Increased LDL-C:* Monitor and treat per standard of care. *Bladder Cancer:* An

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imbalance in bladder cancers was observed in clinical trials. FARXIGA should not be used in patients with active bladder cancer and should be used with caution in patients with a prior history of bladder cancer. *Macrovascular outcomes:* There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with FARXIGA or any other antidiabetic drug. The most common adverse reactions associated with FARXIGA (5% or greater incidence) were female genital mycotic infections, nasopharyngitis, and urinary tract infections [2-9].

Momordica charantia (M. charantia), commonly referred to as bitter gourd, karela and balsam pear. Its fruit is also used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. Abundant pre-clinical studies have documented in the anti-diabetic and hypoglycaemic effects of *Momordica charantia* through various postulated mechanisms. However, clinical trial data with human subjects are limited and flawed by poor study design and low statistical power. The present review is an attempt to highlight the antidiabetic activity as well as phytochemical and pharmacological reports on *M.charantia*and calls for better-designed clinical trials to further elucidate its possible therapeutic effects on diabetes. [10]

There is scope for the potential herb-interactions between *Momordica charantia* and Vildagliptin. This can cause few adverse reactions as a result, it precipitates potentially life-threatening effects. Hence, the studies need to be subjected to pharmacological studies in order to discover their effect on the patients who are taking the treatment with synthetic drugs.

2. Materials and methods:

2.1. Drugs and chemicals

Adult Albino rats weight between 150±20 grams (Mahavear enterprises Hyderabad, Telangana.) were used in this experimental study. These animals were acclimatized to standard laboratory's conditions of suitable temperature (27^{o}C $\pm$ 1^{o}C) and maintained on 12:12 hours light: dark cycle in animal's house. They were maintained in elevated rat's wire cages and provided with regular rat's chow (Standard pellets contains diet – Balaji life sciences Hyderabad, Telangana.), distilled water *ad-libitum* for 14 days.These experimental protocols were in conducted according with IAEC/ CPCSEA.

2.2. Preparation of crude extract

The fresh fruits of *M charantia* (Bitter melon) were purchased from the local market. The fruits were then washed thoroughly with tap water and cut into thin slices. The sliced pieces were dried completely under the mild sun and grinded with an electric grinder into coarse powder and used for cold extraction. After extraction the yield was found to be about 35g/1kg bitter melon powder. The authenticity of *M. charantia*was identified by a plant taxonomist from the Department of Botany, University of Sathavahana University. [11]

2.3. Pre treatment

Albino rats were selected for this study (150±20grams), animals were maintained under the suitable conditions in animal house. [IAEC-1322/po/s/10/CPCSEA].The rats were kept in the animal cages and high fatty food and water are suppled in the form of carbohydrates: proteins: fat in 42:18:40.for 14days.

2.4. Induction of Hyperglycemia in Rats by streptozotocin {60mg/kg}:

After 15 days of feeding with highly fatty food the rats were fasted for a period of 18hrs before the induction of hyperglycemia &singledose administration of the 60 mg/kg of Streptozocin (Sigma Aldrich; St. Louis; MO; USA) were injected intra-peritonially (freshly dissolve in the normal saline solution). After STZ administration, the animals were free accessed with food (pellet diet) & water. moderate polydipsia and marked polyuria were observed in diabetic hyperglycemic rats. After three days i.e. after 72hrs of injection, fasting blood glucose concentration was determined by following glucose levels by using commercial glucose estimation kitswith UV-Visible Spectrophotometer at 505nm based on the oxidase/peroxidase GOD/POD method.The Rats showing the fasting blood glucose level more than 150 mg/dL were considered the hyperglycaemic-rats and selected for the different grouping in the experimental design.

2.5. Experimental Study Design

The hyperglycemic rats were divided in to 6 groups 6 animals in each.

- I Group: Diabetic control
- II Group: Low dose of *Momordica charantia* (100 mg/Kg)

- III Group: High dose of *Momordica charantia* (500 mg/Kg)
- IV Group: Oral hypoglycemic drug- 2 mg/Kg of Dapagliflozin.
- V Group: Combination of 1 mg/Kg of Dapagliflozin+ 500 mg/Kg of *Momordica charantia*
- VI Group: Combination of 2 mg/Kg of Dapagliflozin + 500 mg/Kg of *Momordica charantia* [12].

2.6. Pharmacokinetics study in hyperglycemic rat model:

2.6.1. Single dose Study

These pharmacokinetic studies were carried out in hyperglycaemic rats (weight b/n 180grams and 250 grams). These animals were housed in animal's wire cages with free access to diet and water *ad-libitum.* The overnight fasting rats were divided in to 6 different groups (n=6) and the followed the treatment mention in the study design. Blood samples were collected at predetermined intervals of 0hr,1hr,2hr,4hr,8hr,12hr and 24hr in the hintomicrocentrifugal tubes containing Na^+ citrate from retro-orbital puncture under di ethyl ether anaesthesia. The blood samples were subjectd to centrifugation at 3000 rpm per 10minutes and plasma was stored at -20^{0C} for analysis and estimation of kinetic parameters as AUC 0 - ∞,Cmaxka, ke CL/F, Tmax, V/F, AUC 0-t &$t_{1/2}$.

2.6.2. Multiple dose study

The hyperglycemic rats were dividing into 6 different treatment groups same as mention in study design and daily treatment is carried for 21 days. Samples of blood were collected from different rat's groups on 0^{th},7^{th}, 14^{th}, 21^{st} day immediately after drug treatment. Samples of blood are collected in to microcentrifugal tubes containing Na^+citrate from retro-orbital puncture under anaesthesia. These blood samples were subjected to centrifuged at 3000 rpm per 10 minuts and plasma was stores at -20^0 C for analysis and estimation of kinetic parameters as AUC 0 - ∞, V/F, ka, Cmax ,CL/F, Tmax,ke, AUC 0-t & $t_{1/2}$.

2.7. Pharmacodynamics study in the hyperglycaemic rats

2.7.1. Single dose study

In this study, treatment was given to all groups of animals as per experimental design. Pharmacodynamic parameters like urea, glucose and cholesterol levels were estimated at th interval of 0, 1, 2,4, 8, 12and 24hours by UV spectrophotometer.

2.7.2. Multiple dose study

In this study, daily treatment was given to all groups of animals for 3 weeks as per experimental design. Pharmacodynamic parameter like urea, cholesterol and glucose levels are estimated the time interval of 0, 7, 14and 21 day by UV spectrophotometer.

2.8. Chromatography

Dapagliflozin concentration in plasma samples were estimated by high performance liquid chromatograph Shimadzu HPLC series 1100 and Jasco HPLC PU-2089 equipped with variable wavelength programmable UV or photodiode array detector. This reverse phase HPLC system with C18 column (5 µm particle size; 100 mm length x 4.6 mm diameter) was used as stationary phase. The chromatographic separation was achieved by isocratic mode with a mixture of Acetonitrile: 0.1% Triethylamine (pH-5.0) in the ratio of 50:50v/v as mobile phase. Mobile phase flow rate was 1.0 ml/min and effluent was monitored at 224 nm wavelength.

The calibration curve for Dapagliflozin in rat plasma was linear in concentration range of 10 to 1500 µg/ml (Figure 2). Lower limit of quantification (LLOQ) for Dapagliflozin was 10µg/ml, chromatogram of Dapagliflozin is provided in Figure 3.

2.9. Sample Preparation

To 100µl of serum sample (test or standard) transferred in micro centrifuge tube. To this mixture 200 µl of acetonitrile was added for protein precipitation, resultant mixture was vortexed and centrifuged at 5000 rpm for 5 minutes. Supernatant fluid was filtered through 0.45 µmmembrane filter. Subsequent filtrate (20 µl) was injected in to HPLC for analysis of Dapagliflozin

2.10. Statistical Application

ANOVA followed by Dunnet test was performed for comparison between different groups of animals. *P* value fewer than 5% ($P < 0.05$) was consider the statistically significant. All clinical data were expressed in the form of Mean±Sd. Pharmacokinetics data was calculated by using *pk* solver software and statistical analysis and graphical representations were done by *Graph pad prism-9.0*

3. Results

Table 1 Blood glucose levels mg/dL (0^{th},1^{st} ,2^{nd},4^{th},8^{th}, 12^{th} and 24^{th} Hour) after oral administration of *Momordicacharantia,* Dapagliflozin and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6).

TREATMENT/ HOURS	BLOOD GLUCOSE LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		Dapagliflozin (DOSE)	Dapagliflozin+ M M (DOSE)	
	Vehicle	100 mg/kg	500 mg/kg	2 mg/kg	1 mg/kg +500 mg/kg	2 mg/kg+ 500 mg/kg
0^{th} Hour	334.1 ± 0.11	309.72 ± 0.25*	286.16 ± 0.19**	281.44 ± 0.55**	280.23 ± 0.42**	268.33 ± 0.91**
1^{st} Hour	349.33 ± 0.52	326 ± 0.45*	262.14 ± 0.61**	259.44 ± 0.62**	247.2 ± 0.39**	242.18 ± 0.88**
2^{nd} Hour	354.2 ± 0.43	343 ± 0.52*	227 ± 0.44**	226.51 ± 0.33**	213.22 ± 0.32**	204 ± 0.73**
4^{th} Hour	302.44 ± 1.06	236 ± 0.53*	216.34 ± 0.33**	213.13 ± 0.38**	203.33 ± 0.58**	188.19 ± 0.55**
8^{th} Hour	302 ± 0.41	199.21 ± 1.03*	177.14 ± 0.42**	174.4 ± 0.35**	175.33 ± 0.42**	174.38 ± 0.76**
12^{th} Hour	300.03 ± 0.44	219.63 ± 0.42*	186.3 ± 0.34**	168.2 ± 0.34**	146.79 ± 0.81**	145.28 ± 0.74**
24^{th} Hour	301.18 ± 0.73	224.16 ± 0.48*	218.45 ± 0.63**	175 ± 0.81**	149.1 ± 1.04**	145.16 ± 0.35**

Values are given as mean± Standard deviation.* *Statistical significance $p < 0.001$ & *Statistical significance $p < 0.05$ (compared with the control group)MM- *Momordica charantia* n - number of animals used

Table 2 Blood cholesterol levels mg/dL (0th,1st ,2nd,4th,8th, 12th and 24th Hour) after oral administration of *Momordica charantia* , Dapagliflozin and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6).

TREATMENT/ HOURS	BLOOD CHOLESTEROL LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		Dapagliflozin (DOSE)	Dapagliflozin + M M (DOSE)	
	Vehicle	100 mg/kg	500 mg/kg	2 mg/kg	1 mg/kg + 500 mg/kg	2 mg/kg + 500 mg/kg
0th Hour	205.44 ± 0.72	207.36 ± 0.59	205.9 ± 0.81	209.33 ± 0.55**	199.44 ± 0.51**	193.04 ± 0.75**
1st Hour	202.9 ± 0.15	201.58 ± 0.42	199.42 ± 0.72	196.18 ± 0.75**	182.81 ± 0.71**	183.61 ± 0.56**
2nd Hour	205.35 ± 0.39	188.12 ± 0.38	183.31 ± 0.42	171.13 ± 0.88**	169.18 ± 0.2**	164.15 ± 0.71**
4th Hour	205.14 ± 0.73	179.5 ± 0.66	172.33 ± 0.38	159.44 ± 0.51**	152.61 ± 0.44**	147.19 ± 0.33**
8th Hour	209.22 ± 0.41	149.01 ± 0.36	146.5 ± 0.38	148.54 ± 1.33**	136.19 ± 1.08**	139.09 ± 0.55**
12th Hour	213.16 ± 0.76	159.05 ± 0.61	159.18 ± 0.81	145.59 ± 0.5**	133.19 ± 0.72**	128.14 ± 0.61**
24th Hour	216.42 ± 0.43	178.04 ± 0.83	170.35 ± 1.99	134.61 ± 0.82**	131.18 ± 0.93**	128.12 ± 0.73**

Values are given as mean± Standard deviation.* *Statistical significance p < 0.001 &*Statistical significance p < 0.05 (compared with the control group)MM- *Momordica charantia* n - number of animals used

Table 3 Blood urea levels mg/dL (0th,1st ,2nd,4th,8th, 12th and 24th Hour) after oral administration of *Momordica charantia* , Dapagliflozin and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6).

TREATMENT/ HOURS	BLOOD UREA LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		Dapagliflozin (DOSE)	Dapagliflozin + M M (DOSE)	
	Vehicle	100 mg/kg	500 mg/kg	2 mg/kg	1 mg/kg + 500 mg/kg	2 mg/kg + 500 mg/kg
0th Hour	64.6 ± 0.81	63.9 ± 0.71	71.16 ± 0.66	76.25 ± 0.44	66.72 ± 0.51	63.16 ± 0.33
1st Hour	63.42 ± 0.44	62.13 ± 0.41	68.41 ± 0.51	65.13 ± 1.41	61.4 ± 0.76	56.8 ± 0.39
2nd Hour	68.13 ± 0.58	62.36 ± 0.44	65.23 ± 0.38	64.26 ± 0.58	55.19 ± 0.44	51.18 ± 0.49
4th Hour	70.44 ± 0.36	61.69 ± 0.44	64.91 ± 0.18	61.51 ± 0.38	48.22 ± 0.58	46.18 ± 0.29
8th Hour	71.21 ± 0.91	58.18 ± 0.62	45.6 ± 0.91	48.19 ± 0.84	44.2 ± 0.72	40.84 ± 0.73

12th Hour	71.83 ± 0.82	61.18± 0.74	57.89 ± 0.61	48.36 ± 0.71	41.19 ± 0.92	38.53 ± 0.52
24th Hour	70.79 ± 0.88	62.44 ± 0.59	58.10 ± 0.82	38.45 ± 0.11	39.19 ± 0.56	34.83 ± 0.44

Table 4 Blood glucose levels mg/dL (0th,7th, 14th and 21st day) after oral administration of Dapagliflozin, and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6)

TREATMENT/ DAYS	**BLOOD GLUCOSE LEVELS (mg/dL)**					
	DIABETIC CONTROL	**MM (DOSE)**		**Dapagliflozin (DOSE)**	**Dapagliflozin + M M (DOSE)**	
	Vehicle	**100 mg/kg**	**500 mg/kg**	**2 mg/kg**	**1 mg/kg +500mg/kg**	**2 mg/kg + 500 mg/kg**
0th Day	310.31 ± 1.01	218.90 ± 0.61	292.14 ± 0.76	301.18 ± 0.69	381.18 ± 0.75	372.14 ± 0.66
7th Day	292.88 ± 0.65	194.88 ± 0.54	193.6 ± 0.66	209.04 ± 0.44	213.18 ± 0.94	197.51 ± 1.09
14th Day	289.39 ± 0.44	182.55 ± 0.52	182.18 ± 1.04	184.18 ± 2.09	128.03 ± 0.61	129.18 ± 0.33
21st Day	291.143± 1.03	145.94 ± 0.3	104.63 ± 0.41	115.08 ± 0.55	113.22 ± 0.58	111.49± 0.22

Table 5 Blood cholesterol levels mg/dL (0th,7th, 14th and 21st day) after oral administration of *Momordica charantia* , Dapagliflozin and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6).

TREATMENT/ DAYS	**BLOOD CHOLESTEROL LEVELS (mg/dL)**					
	DIABETIC CONTROL	**MM (DOSE)**		**Dapagliflozin(DOSE)**	**Dapagliflozin + M M (DOSE)**	
	Vehicle	**100 mg/kg**	**500 mg/kg**	**2 mg/kg**	**1 mg/kg + 500 mg/kg**	**2 mg/kg + 500 mg/kg**
0th Day	194.73 ± 0.91	189.9 ± 0.72	186.84 ± 0.91	185.91 ± 0.22	183.61 ± 1.04	176.11 ± 1.05
7th Day	195.33 ± 1.28	105.74 ± 0.22	105.18 ± 0.44	119.94 ± 0.33	98.33± 0.41	92.55 ± 0.94
14th Day	192.4 ± 0.46	89.18 ± 0.66	86.41 ± 0.59	85.44 ± 0.81	76.91 ± 0.82	78.55 ± 0.66
21st Day	188.34 ± 0.54	78.43 ± 0.86	67.17 ± 0.52	68.28 ± 0.18	61.53 ± 0.29	58.22 ± 0.29

Table 6 Blood urea levels mg/dL (0th,7th, 14th and 21st day) after oral administration of *Momordica charantia* , Dapagliflozin and combination of Dapagliflozin and *Momordica charantia* in diabetic rats (n=6).

TREATMENT/ DAYS	BLOOD UREA LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		Dapagliflozin (DOSE)	Dapagliflozin + M M (DOSE)	
	Vehicle	100 mg/kg	500 mg/kg	2 mg/kg	1 mg/kg + 500 mg/kg	2 mg/kg + 500 mg/kg
0th Day	69.11 ± 0.35	65.38 ± 0.33	67.15 ± 0.55	64.31 ± 0.39	68.28 ± 0.17	58.28 ± 0.38
7th Day	76.43 ± 0.48	45.19 ± 0.48	40.49 ± 0.35	34.38 ± 0.66	32.58 ± 0.62	26.37 ± 0.69
14th Day	78.49 ± 0.22	36.18 ± 0.59	34.11 ± 0.18	29.18 ± 0.99	23.96 ± 0.81	22.64 ± 0.83
21st Day	84.32 ± 0.73	34.25 ± 0.54	29.45 ± 1.19	24.19 ± 0.63	21.46 ± 0.18	19.88 ± 0.66

Table 7 Mean plasma Dapagliflozin concentrations (µg/ml) (Single dose study)

TREATMENT/ Hours	PLASMA CONCENTRATIONS (µg/ml)			
	DIABETIC CONTROL	Dapagliflozin (DOSE)	Dapagliflozin + M M (DOSE)	
	Vehicle	2 mg/kg	1 mg/kg +500mg/kg	2 mg/kg +500 mg/kg
1st Hour	0	51.65±0.011	35.16±0.025	42.15±0.023
2nd Hour	0	81.64±0.022	64.99±0.026	72.34±0.019
4th Hour	0	62.59±0.044	42.31±0.031	50.19±0.034
8th Hour	0	51.28±0.023	30.48±0.022	34.66±0.042
12th Hour	0	21.33±0.019	14.81±0.021	18.63±0.052
24th Hour	0	0	0	0

Table 8 Mean plasma Dapagliflozinconcentrations (µg/ml) (Multiple dose study).

TREATMENT/ DAYS	PLASMA CONCENTRATIONS (µg/ml)			
	DIABETIC CONTROL	Dapagliflozin(DOSE)	Dapagliflozin + M M (DOSE)	
	Vehicle	2 mg/kg	1 mg/kg + 500 mg/kg	2 mg/kg + 500 mg/kg
0th Day	0	56.18±0.022	39.62±0.052	43.16±0.036
7th Day	0	78.26±0.053	45.18±0.014	61.03±0.015
14th Day	0	44.18±0.032	31.82±0.028	40.91±0.013
21st Day	0	32.18±0.023	16.32±0.018	19.16±0.025

Table 9 Effect of *Momordica charantia* on Pharmacokinetic parameters of Single dose administration of Dapagliflozin in diabetic rats (n=6).

Pharmacokinetic parameter	**Units for Pharmacokinetic parameters**	**2 mg/kg of Dapagliflozin**	**Dapagliflozin + *Momordica charantia* (DOSE)**	
			1 mg/kg+ 500 mg/kg	**2 mg/kg+ 500 mg/kg**
ka	h^{-1}	1.920±0.29	1.821±0.33	1.861±0.29
ke	h^{-1}	0.682±0.33	0.688±0.16	0.691±0.24
t1/2	h	12.9±1.31	12.1±0.13	12.5±1.19
V/F	(mg/kg)/(µg/ml)	118.19±14.52	100.26±9.04	109.18±10.19
CL/F	(mg/kg)/(µg/ml)/h	3.51 ± 4.33	3.53 ± 3.44	3.59± 3.83
Tmax	h	2.4±0.1	2.1±0.1	2.2±0.1
Cmax	µg/ml	1204 ± 180	1186 ± 142	1199 ± 161
AUC 0-t	µg/ml*h	3150 ± 64.30	2819 ± 23.06	2950 ± 39.04
AUC 0 - ∞	µg/ml*h	3328 ± 63.02	3042 ± 53.08	3181 ± 61.28

Table 10 Effect of *Momordica charantia* on Pharmacokinetic parameters of Multiple dose administration of Dapagliflozin in diabetic rats (n=6)

Pharmacokinetic parameter	**Units for Pharmacokinetic parameters**	**2 mg/kg of Dapagliflozin**	**Dapagliflozin + *Momordica charantia* (DOSE)**	
			1 mg/kg+ 500 mg/kg	**2 mg/kg+ 500 mg/kg**
ka	h^{-1}	1.932±0.18	1.900±0.28	1.916±0.44
ke	h^{-1}	0.686±0.42	0.687±0.25	0.689±0.32
t1/2	h	12.5±1.26	12.2±0.14	12.4±1.07
V/F	(mg/kg)/(µg/ml)	116.24±10.93	109.34±8.91	114.13±10.22
CL/F	(mg/kg)/(µg/ml)/h	3.53 ± 3.21	3.54 ± 2.19	3.56± 2.93
Tmax	h	2.6±0.1	2.4±0.1	2.5±0.1
Cmax	µg/ml	1201 ± 152	1188 ± 166	1194± 132
AUC 0-t	µg/ml*h	3144 ± 42.93	2855 ± 19.85	2914± 38.91
AUC 0 - ∞	µg/ml*h	3290 ± 45.02	3099 ± 44.52	3190 ± 58.06

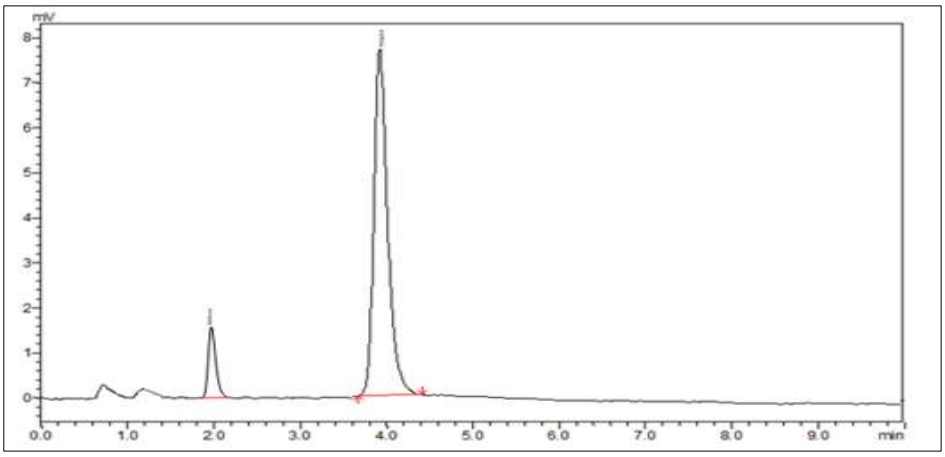

Figure 1 Chromatogram of Dapagliflozin

Table10 Calibration curve of Dapagliflozin

Calibration curve of Dapagliflozin	
Concentration (g/mL)	Peak area
10	500
50	1800
80	2800
100	4600
120	5800
150	7000
500	21000
1500	80000

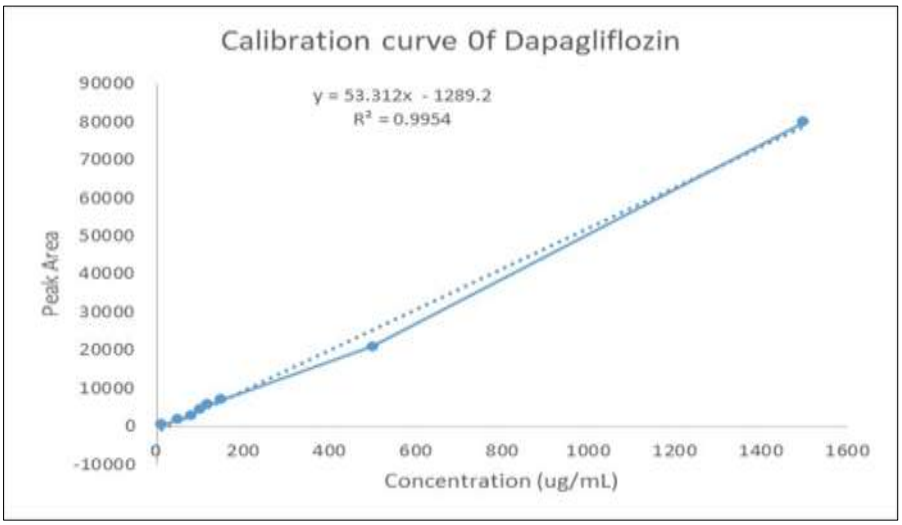

Figure 2 Calibration curve of Dapagliflozin

4. Discussion

4.1. Pharmacodynamic study

The combination of high dose of Dapagliflozin (2 mg/kg) with 500 mg/kg *Momordica charantia* showed maximum hypoglycemic action, decrease in serum cholesterol and urea levels. The effect produced by combination of Dapagliflozin (1 mg/kg) with *Momordica charantia* was greater than the hypoglycaemic action produced by *Momordica charantia* (500 mg/kg) alone and Dapagliflozin (2 mg/kg).Blood cholesterol and Urea reduction were also high in combination group compared to individual Dapagliflozin and *Momordica charantia* groups alone.

4.2. Pharmacokinetic study

4.2.1. Single dose study

It was observed that AUC(0 - ∞) was decreased by 8.6% in 500 mg/kg of*Momordica charantia* and 1 mg/kg of dapagliplozin, while 4.5% in 500 mg/kgof *Momordica charantia* and 2 mg/kg of dapagliplozin.Cmax was decreased by 1.5% in 500 mg/kg of *Momordica charantia* and1 mg/kg of dapagliplozin, 0.5% in 500 mg/kg of *Momordica charantia* and2 mg/kg of dapagliplozin in single dose study. There was decrease in Absorption rate constant Ka by 5.16% in 500 mg/kg of*Momordica charantia* and 1 mg/kg of dapagliplozin, 3% in 500 mg/kg of *Momordica charantia* and 2mg/kg of dapagliplozin.It was found that slight increase in Clearance in 500 mg/kg of *Momordica charantia* with 1 mg/kg of dapagliplozin and in 500 mg/kg of *Momordica charantia* with 2 mg/kg of dapagliplozin.

4.2.2. Multiple dose study

It was observed that AUC(0 - ∞) was decreased by 6% in 500 mg/kg of *Momordica charantia* and 1 mg/kg of dapagliplozin, while 3.1% in 500 mg/kg of *Momordica charantia* and 2 mg/kg of dapagliplozin . C max was decreased by 1.1% in 500 mg/kg of *Momordica charantia* and 1 mg/kg of dapagliplozin, 0.5% in 500 mg/kg of *Momordica charantia* and 2 mg/kg of dapagliplozin in single dose study. There was decrease in Absorption rate constant Ka by 5.16% in 500 mg/kg of *Momordica charantia* and 1mg/kg of dapagliplozin, 3% in 500 mg/kg of *Momordica charantia* and 2 mg/kg of dapagliplozin. It was found that slight increase in Clearance in 500 mg/kg of Momordica charantia with 1mg/kg of dapagliplozin and in 500 mg/kg of Momordica charantia with 2 mg/kg of dapagliplozin.

5. Conclusion

The above herb-drug interaction reveals that combination of Dapagliflozin (2 mg/kg) with 500 mg/kg of *Momordica charantia* have hypoglycemic action significantly as compared to the Dapagliflozin (1 mg/kg) with *Momordica charantia* (500 mg/kg) and Dapagliflozin (2 mg/kg) alone in pharmacodynamic studies.Combination of Dapagliflozin (2 mg/kg) with 500mg/kg of *Momordica charantia* have increased AUC, C max , absorption rate constant Ka and decreased clearance as compared to the Dapagliflozin (1 mg/kg) with *Momordica charantia* (500 mg/kg) in pharmacokinetic studies.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

All experimental process of animals was approved by the Institutional Animal Ethical Committee of SRR college, of pharmacy.

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