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BIOANALYTICAL METHOD DEVELOPMENT OF METFORMIN CO-ADMINISTERED WITH *OCIMUM SANCTUM* FOR POTENTIAL BIOENHANCER ACTIVITY

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ABSTRACT: Objective: The present study was an effort to evaluate the effect of *Ocimum sanctum* on plasma concentration of metformin HCl and investigating its pharmacokinetic parameters using bioanalytical method. **Methods:** Seven groups comprising of six rats were used. Group I served as normal control and Group II was negative control of Streptozotocin induced Diabetic rats. Group III received 100mg/kg p.o. Metformin alone while Group IV and V were administered with 100mg/kg and 150mg/kg p.o. extract respectively. Group VI and VII were given metformin and extract in combination. Blood glucose level was determined in treated rats for 0.5, 2, 4, 8, 12 and 24hr as part of protocol I and directly on 2nd, 4th and 7th day as part of protocol II. RP-HPLC method was developed and validated for estimation of plasma levels of metformin HCl in plasma. With the help of above developed method, pharmacokinetic parameters were evaluated in plasma for metformin alone and combination with *Ocimum sanctum*. **Results:** RP-HPLC method was developed successfully for determination of plasma concentration of metformin HCl. *Ocimum sanctum* extract (100mg/kg) with metformin HCl (100mg/kg) shows higher Cmax (19.8ng/ml) and Tmax shifted to lower side (3.027hrs) as compared with 100mg/kg of metformin alone indicating improvement in plasma concentration of metformin. **Conclusion:** The present study indicates that the methanolic extract of *Ocimum sanctum* may have antihyperglycemic activity. It also improves availability of metformin. Thus dose or frequency of dosing of metformin can be minimized and *Ocimum sanctum* may act as bioenhancer for metformin when administered orally.

INTRODUCTION: The use of herbs as drugs is a growing trend especially in the elderly for the management of chronic aliment. Most of chronic diseases are difficult to treat successfully with orthodox drugs. Diabetes mellitus is major health concern and most common endocrine disorder with deranged carbohydrate, fat and protein metabolism resulting from total or relative insulin deficiency (insulin dependent diabetes mellitus) or resistance to insulin action (non-insulin dependent diabetes mellitus).

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This chronic aliment has been acclaimed to be managed by traditional healers with over 400 plants reported to have antidiabetic properties including *Eugenia jambolana, Mucuna pruriens, T. cordifolia, T. foenum graecum, Ocimum sanctum* and *Brassica juncea* [1, 2].

The objective of this study is to find out whether the *Ocimum sanctum* leaf extract helps in enhancing the plasma concentration for Metformin and thus helping in increasing bioavailability of drug. Study was planned to check bioenhancer activity for metformin by carrying out various *in-vitro* and *in-vivo* study with plasma estimations using LC method.

Ocimum sanctum is found throughout India. The leaves of *Ocimum sanctum* have shown to cause

significant reduction of blood glucose level in normal, glucose fed hyperglycemic and STZ induced diabetic rats. The plant has also demonstrated anti-oxidant and hypolipidimic effect [3]. In present study, the *Ocimum sanctum* leaves are selected as a candidate herbal drug to check whether it has enhancing effect on plasma concentrations of Metformin, a potential antidiabetic drug. Thus, it may be evaluated for its role in bio enhancement. The herbal drug may show hypoglycemic activity itself and hence may act as synergistic agent for synthetic dug. It may, otherwise, improve plasma concentration of synthetic drug and thus help in lowering dose/ frequency of dosing of synthetic drug when used in combination with synthetic drug.

Metformin ($C_4H_{11}N_5$) is 1,1-dimethylbiguanidine is an anti-diabetic drug from the biguanide class of oral hypoglycemic agents, given orally in the treatment of non–insulin-dependent diabetes mellitus. There are many antidiabetic molecules used in conventional therapies for diabetes. Metformin is one of the most highly preferred drug. But it has limitation that it is negligibly bound to plasma proteins. In addition, its bioavailability is only 50-60% [4]. Hence we can employ a herbal drug which may in combination with synthetic drug, show better results.

The RP-HPLC method for the quantification of metformin in pure form and in plasma has been developed [5, 6].

Bioanalytical methods are widely used to quantitate drugs and their metabolites in physiological matrices, and the methods could be applied to studies in areas of human clinical pharmacology and nonhuman pharmacology/toxicology. One of the major challenges facing the pharmaceutical industry today is finding new ways to increase productivity, decrease costs whilst still ultimately developing new therapies that enhance human health. Bioanalytical method employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK) and toxicokinetic studies. Since plasma is one of the most widely adopted biological fluid in drug discovery and development, the focus of this discussion will be limited to plasma analysis. The steps in bioanalytical methods are method development, method validation and sample analysis [7, 8].

2. MATERIALS AND METHODS:

2.1 Drugs and reagents:

Metformin HCl was gifted by Cipla Pvt. Ltd., Mumbai, India. Acetonitrile and methanol of HPLC grade were from Merck Specialties and Loba Chemie Pvt. Ltd., Mumbai, India respectively. Streptozotocin (STZ) was purchased from Siaco Research Laboratories Pvt. Ltd. Mumbai, India. Other chemicals used in this study were all of analytical grade unless specified.

2.2 Plant material:

Fresh leaves of *Ocimum sanctum* green variety were collected from the local market, Pune,India. The plant material was authenticated by Botanical survey of India (BSI), Pune. The leaves were shade dried at room temperature. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then defatted by n-hexane solvent for 24hrs by maceration. The marc was dried and further extracted with methanol. The cold maceration was continued for 24hrs. The obtained extract was concentrated using rotary evaporator and dried using a vaccum oven, yielding 5.5gm of extract (3.81%w/w). The extract was subjected to phytochemical analysis [9].

2.3 Animals:

Sprague Dawley rats females, weighing 200-250 g were procured from National Institute of Bioscience, Pune. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/2013/14/166) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune, constituted under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were placed in polypropylene cages (six per cage) randomly with paddy husk as bedding. Animals had free access to water and standard laboratory feed (Amrut feed, Chakan, India). The animals were shifted from animal house

to the laboratory one hour prior to the start of the experiment.

2.4 Method Development and validation of Metformin in plasma:

The RP-HPLC study was carried out using Shimadzu LC 2010CHT, Shimatzu, Japan. The mobile phase was prepared by mixing 0.1 M monobasic ammonium phosphate (pH 3.1) and acetonitrile in the ratio of 60:40; followed by filtration using 0.22 µ membrane filter. The elution was carried out on (BDS HYPERSIL C18 column) at a flow rate of 1 ml/min.

Detection of metformin was carried out at 233 nm at 40^{o}C. 1.5ml of blood was collected from retro orbital plexus of Sprague Dawly Rats and then centrifuged at 3000 rpm for 20 min to separate the plasma. From 50µg/ml of stock solution 100 µl amount was added to 500 µl plasma and vortexed for 60 sec. Then 1ml of acetonitrile was added and vortexed for 1 min and centrifuged for 10 min at 3000 rpm. Then, supernatant was separated, evaporated, reconstituted with mobile phase and analyzed on HPLC [6].

2.4.1: Method validation [10]

- **Preparation of Working Standard Solution of Metformin for Calibration Curve:**

100 µg/ml solution was prepared by diluting 1ml of stock solution (1000 µg/ml) to 10 ml. Just prior to spiking, working standard solution of analyte were prepared from this stock solution, to get the concentration range of 0.18 µg/ml to 24µg/ml. Methanol was used as diluent. The linearity of method was checked.

- **Preparation of spiked plasma Dilutions for Calibration Curve of Metformin:**

Transferred 100µl of the above described working solution into 500µl plasma to achieve the concentration ranging from 37.5ng/ml to 4800 ng/ml. The linearity of method was checked.
(The spiked Plasma samples used for study were stored at -20°C until analysis)

- **Preparation of spiking plasma Dilution for Quality Control Samples of Metformin:**

Transferred 100µl of the above described working solution into vials and added 500µl plasma to achieve the concentrations ranging from 50ng/ml to 4000ng/ml and labeled them as LOQ, MQC, and HQC respectively

I) Accuracy and Precision:

The intra and inter-day precision and accuracy determined by percent coefficient of variation (%C.V) and percent nominal values. Solutions containing lowest, intermediate, and highest concentrations solution of the calibration curve, i.e. 50, 1000, and 4000 ng/ml. six injections at each of the specified concentration levels injected within the same day for repeatability.

II) Selectivity:

The selectivity is generally defined as the lack of interfering peaks at the retention time of the assayed drugs in the chromatograms. Blank samples of the appropriate biological matrixes were obtained from six different sources. Then these samples were analyzed along with LLOQ samples.

III) Sensitivity:

Sensitivity is measured in terms of LLOQ. Prepared six plasma spiked LLOQ samples using the same spiking dilution. These six LLOQ samples were processed and tested along with calibration curve standards in same range used for calculation of precision and accuracy.

IV) Stability:

Bench top stability is carried to assess the stability of the analyte in biological fluids over a period of time during which the samples are expected to be kept on the bench while processing. For Bench top stability, six sets each of LQC, MQC and HQC from the deep freezer were withdrawn and then they were left at room temperature for at least 6 hrs.

2.5 Pharmacokinetic study:

2.5.1 Oral Glucose Tolerance Test (OGTT) [11]:

Antihyperglycemic activity was studied in glucose-loaded hyperglycemic rats. Metformin (100mg/kg) was used as standard. Group I was kept as negative control and received only vehicle. The remaining groups of rats were treated with 100mg/kg and 150mg/kg of methanolic extract. Blood sugar level was determined in overnight fasted animals at 0 hours. After 30 mins of the drug treatment, animals

were fed with glucose (4g/kg) and blood glucose was determined at 1/2, 1, 2, and 3 hours after glucose load. Blood glucose concentration was estimated by using a commercial glucometer and test strips by collecting blood from the tail vein.

2.5.2 Induction of Diabetes mellitus in rats for study [12]:

A rat model of Type II Diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) was induced in overnight-fasted rats by a single intraperitoneal injection of Streptozotocin (60mg/kg). Blood samples were obtained from the retro-orbital plexus in both Streptozotocin injected and control animals. Hyperglycemia was confirmed by elevated blood glucose levels determined at 72 hrs. The rats were supplied with 10% glucose water and feed during the next 24 hours to avoid sudden hypoglycemia post-injection. On day 2, glucose water was replaced with drinking water. Fasting blood glucose levels were determined by glucometer. Rats with fasting blood glucose levels above 250 mg/dL were considered diabetic.

2.5.3 Animal grouping and experimental protocol:

The diabetic animals were assigned to into seven groups of 6 rats each.

Group-I: Normal Control

Group-II: Diabetic Control

Group-III: Metformin 100 mg/kg, p.o.

Group-IV: Methanolic Extract 100 mg/kg, p.o.

Group-V: Methanolic Extract 150 mg/kg, p.o.

Group-VI: Methanolic Extract 100 mg/kg+ Metformin 100 mg/kg, p.o.

Group-VII: Methanolic Extract 150 mg/kg+ Metformin 100 mg/kg, p.o.

2.5.4 Methodology:

The animals were grouped randomly each having six animals. The control group received Vehicle and in the treatment group the drug/extracts were administered orally during the duration of study.

Protocols: [1, 13]

1) One day Protocol: The rats were fasted overnight. The drug/extract were administered to respective groups orally as given below and blood samples were withdrawn from retro orbital plexus at 0.5, 2, 4, 8, 12, and 24 hrs to check the Plasma concentration of metformin and to determine pharmacokinetic parameters for the same.

2) Seven day Protocol:

The rats were fasted overnight. The drug/extract were administered to respective groups orally as given below. The blood samples were withdrawn from tail by tail vein method at intervals of 24hrs, 72hrs, and 168hrs to check the Plasma concentration of metformin and to determine pharmacokinetic parameters for the same.

In both the protocols, pharmacokinetic parameter was determined by area analysis. By plotting area of sample chromatogram in linearity equation the concentration of each plasma sample was calculated. The plasma metformin concentration–time data was put in to Microsoft pka software and Area Under Curve (AUC) was calculated.

2.5.5 Determination of blood glucose:

Glucometer (Contour-TS) was used for the determination of blood glucose levels of the rats. Blood samples were obtained from tail-vein method. The blood glucose levels were recorded using glucose test strip.

2.5.6 Preparation and Analysis of rat plasma Samples:

Analytes were extracted from rat plasma by protein precipitation using acetonitrile. Samples were collected in Eppendorf's tube. And vortexed for 5 min. Samples were centrifuged for 10 min. at 3000rpm to separate other contents of blood from plasma. 1.5ml acetonitrile was added in each sample and vortexed for 1 min. All samples then centrifuged for 10 min at 3000 rpm. Then, 500µl supernatant was separated, evaporated, reconstituted with mobile phase and analyzed on HPLC and pharmacokinetic parameters were observed [6].

3. RESULTS:

The drug was authenticated as *Ocimum sanctum* by Botanical Survey of India, Pune and specimen no. (AS 01) was allotted to sample.

3.1 Preliminary phytochemical screening of methanolic extract of *Ocimum sanctum* leaves:

The phytochemical studies showed the presence of mainly Alkaloids, Carbohydrates, Glycosides and Tannins. (**Table 1**)

TABLE 1: PHYTOCHEMICAL TEST OF METHANOLIC EXTRACT OF *OCIMUM SANCTUM* LEAVES

No	Phytochemical constituent	Test	MEOS
1.	Alkaloid	Mayer's reagent	+
		Wagner's reagent	+
2.	Carbohydrate	Molisch's reagent	+
		Fehling's reagent	+
		Barfoed's reagent	+
		Benedict's reagent	+
3.	Glycosides	Borntrager's reagent	+
4.	Protiens& Amino acids	Millon's reagent	+
		Biuret's reagent	+
5	Steroids	Liebermann Buchard test	-
		Salkowski test	+
6.	Cardiac Glycoside	Baljet test	-
7.	Tannins	5% $FeCl_3$ reagent	+
		Lead acetate reagent	+

Note: Present (+), Absent (-)
MEOS - Methanolic Extract of *Ocimum sanctum*

3.2 Thin layer chromatography of extracts and eugenol [14]

TLC was developed in mobile phase Toluene: Ethyl acetate (93:7) (**Fig. 1**).

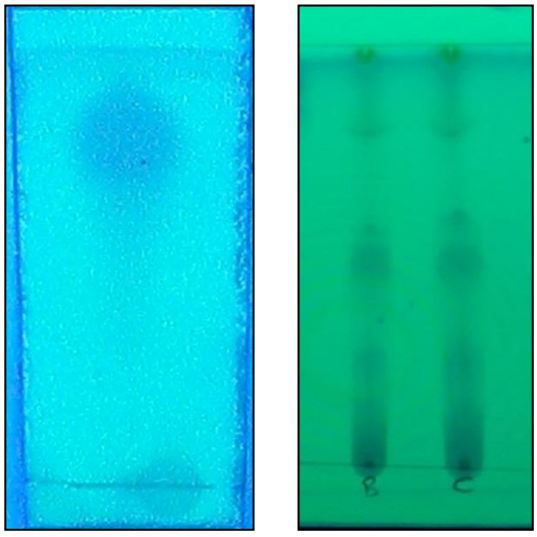

FIG.1: THIN LAYER CHROMATOGRAPH OF *OCIMUM SANCTUM* EXTRACTS AND EUGENOL

The R_f of the spot of extracts matches with the Rf of eugenol used as standard (**Table 2**).

TABLE 2: R_f VALUES OF EXTRACT AND EUGENOL

No.	Type of Extract	Rf observed value	Rf Literature value
a)	Eugenol	0.70	Eugenol 0.79
b)	Methanolic extract	0.2	Essential oils
		0.5	0.2-0.4
		0.8	Caryophylline 0.5 Eugenol 0.79

3.3 Optimization of HPLC Method for Plasma Spiked Metformin:

In the present method, acetonitrile was used for deproteinization of plasma and it has effectively deproteinized the plasma and hence, there was no necessity of separately using acid for this purpose. When metformin extracted by acetonitrile from plasma was injected onto C18 column using 0.1 M monobasic ammonium phosphate buffer (pH 3.1) and acetonitrile in the ratio of 60:40 as mobile phase, metformin at 2.5 min with good linearity indicating the preciseness and accuracy of the developed method (**Fig.2**).

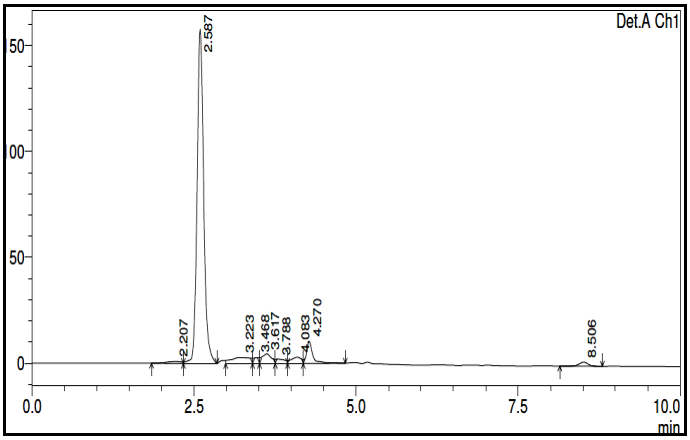

FIG. 2: CHROMATOGRAM OF PLASMA SPIKED WITH METFORMIN

3.4: Method Validation:

3.4.1 Calibration Curve of Metformin:

Linearity of Metformin was evaluated by determining eight standard working solutions containing 0.18-24 µg/ml which showed good correlation coefficient. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient at λ max of 233nm (**Fig. 3**).

3.4.2 Calibration curve of Plasma spiked Metformin:

Metformin linearity was established in the range of 37.5ng/ml to 4800ng/ml. Correlation coefficients

(r^2) were greater than 0.99 which meets the acceptance criteria (**Fig. 4**).

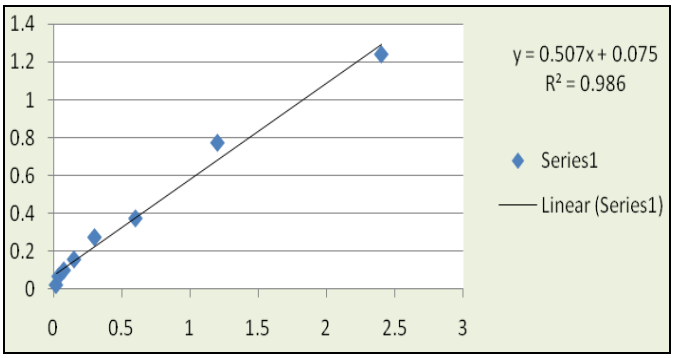

FIG.3: CALIBRATION CURVE OF METFORMIN IN METHANOL

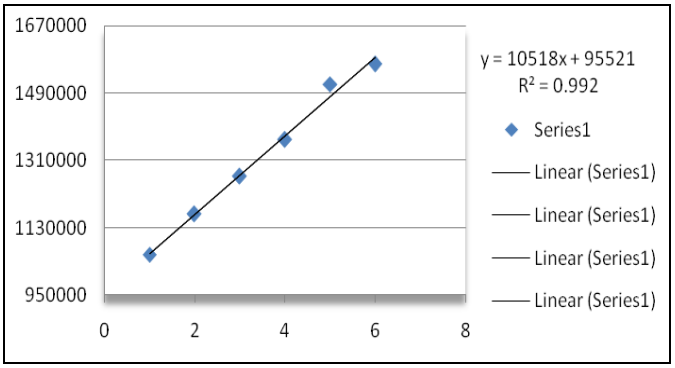

FIG.4: CALIBRATION CURVE OF PLASMA SPIKED METFORMIN

3.4.3 Precision and Accuracy:

For LQC, MQC, & HQC, the intra-day precision was 4.27%, 14.41% and 16.06%, and the intra-day accuracy 98.80%, 111.92% and 107.60%, respectively [10].

For LQC, MQC & HQC, the inter-day precision was 6.38%, 0.83% and 23.25%, and the interday accuracy 114.72%, 118.03% and 88.43%., respectively. The calculated values of accuracy and precision (**Table 3**), Selectivity is expressed in terms of % interference. The response of interference peak at retention time of Metformin was found to be 0% [10].

Sensitivity **Table 4** and stability **Table 5** of the method were well in accordance with the USFDA guidelines for bioanalytical method validation [10].

3.5 Pharmacokinetic study:

3.5.1 Oral Glucose Tolerance test:

The glucose tolerance test was conducted on normal rats fed with Metformin and methanolic extract of *Ocimum sanctum*, the results found decrease in blood glucose levels (**Table 6**).

TABLE 3: PRECISION AND ACCURACY

	Known Conc.(ng/ml)	Mean conc.±S.D. (ng/ml)	Accuracy (%)	Precision (%CV)
Intraday	50	49.40±2.11	98.80%	4.27
	1000	1119.21±161.32	111.92%	12.41
	4000	4304.26±691.62	107.60%	14.06
Interday	50	57.36±3.66	114.72%	6.38
	1000	1180.30±9.86	118.03%	0.83
	4000	3137.45±729.76	88.43%	13.25

Each value is mean of 6 replicates; S.D. - Standard deviation; CV- Coefficient of variation

TABLE 4: SENSITIVITY

Known Conc.(ng/ml)	Mean conc.±S.D. (ng/ml)	(%) Nominal	(%CV)
37.5	37.45±0.41	99.86	1.09

TABLE 5: STABILITY

Known Conc.(ng/ml)	Mean conc.±S.D. (ng/ml)	(%) Nominal	(%CV)
50	47.11±1.76	94.20%	3.73
1000	981.90±5.89	98.18%	0.60
4000	3951.28±28.44	98.78%	0.71

TABLE 6: ORAL GLUCOSE TOLERANCE TEST (OGTT)

Treatment	Dose mg/kg	Blood Glucose level (mg/dl) at time t (hrs.)					
		0		1/2	1	2	3
Glucose Control	-	62.67±2.011	Glucose Load 4 mg/kg	126.5±2.57	144±2.868	109.7±3.18	101±2.556
Metformin	100	63.33±1.498		87.67±2.61*	77.83±1.92*	70.17±1.4*	61.33±1.05*
Methanolic OS	100	63±1.498		90.83±1.868*	75.33±1.87**	65.17±1.276**	58.83±0.87**
Methanolic OS	150	57.5±1.176		88±2.113*	80.33±1.64	69±1.826**	56.5±1.54**

OS- *Ocimum sanctum*

The values are expressed as mean ± SEM, n = 6 , *p<0.05, **p<0.01 when compared to Glucose Control**.**

3.5.2 Blood glucose levels for 24hrs and 7 day study:

The blood sugar level of the Metformin and combination with extract of *Ocimum sanctum* showed significant difference at 24hrs Protocol (p<0.05 and p<0.01, respectively), when compared to the Metformin (**Table 7**). In 7 day protocol all treatment groups showed significant variations compared with Metformin (**Table 8**).

TABLE 7: BLOOD GLUCOSE LEVELS FOR 24HRS. STUDY

Treatment	0 hr	1/2 hr	2 hrs	4 hrs	8 hrs	12 hrs	24 hrs
Metformin	383.3±3.981	367.3±3.528	343.5±3.739*	321.8±2.548	302±2.517**	299.8±3.103	271.32.512**
OSM I	366.8±3.683	354.8±2.915	336.7±3.211*	324.7±2.871**	319±3.246**	310±4.008**	267.5±3.452**
OSM II	357.8±3.557	352.2±3.719	332.5±2.012*	327.7±2.14	322.2±1.99	317.5±3.128*	283.8±2.574*
Normal	65.67±2.333	66.5±2.579	70.33±2.216	67.5±1.232	68.5±0.6191	68.5±0.8466	69.17±0.83
STZ	372±5.627	374.8±4.813	375.3±6.059	381.5±3.528	385.7±2.525	376.5±2.825	351±2.16

TABLE 8: BLOOD GLUCOSE LEVELS FOR 7 DAY STUDY

Groups	1 day	2 day	4 day	7 day
Normal	65.67±2.333	69.17±0.83	73±3.276	75.5±3.096
STZ	372±5.627	351±2.16	249.5±4.161	198.3±4.03
Metformin	383.3±3.981	271.32.512**	173±2.394**	141.2±3.005**
OS I	371.2±5.77	292.3±2.486*	220.3±3.201*	165±3.587*
OS II	369±4.872	259.2±2.892*	207.7±5.432*	162.2±3.486**
OSM I	366.8±3.683	267.5±3.452**	174.5±3.354**	126.7±2.376**
OSM II	357.8±3.557	283.8±2.574	184.3±3.63**	137.32.404**

OS- *Ocimum sanctum*; OSM- *Ocimum sanctum*+ metformin; STZ- Streptozotocin

3.5.4 Protocols:

The pharmacokinetic parameters for plasma samples were determined.

I) One day protocol: It indicates the Cmax of plain metformin is 15.96ng/ml and Tmax 4 hrs. with AUC 156.09 (**Table 9**).

TABLE 9: PHARMACOKINETIC PARAMETERS (METFORMIN) AT 24HR STUDY

Sr No.	Parameters	Results		
		Metformin (100mg/kg)	Metformin(100mg/kg)+ *Ocimum sanctum* 100mg/kg	Metformin 100mg/kg)+ *Ocimum sanctum* 150mg/kg
1	Area Under Curve	156.09	219.075	194.66
2	Half-life t1/2 (hr.)	2.23	2	3.51
3	Cmax (ng/ml)	15.96±1.066	19.8±0.825	18.76±0.328
4	Tmax (hr.)	4	3.027	3.51

While the combination of metformin with methanolic extract of *Ocimum sanctum*, show Cmax of 19.8ng/ml and Tmax 3.027 hrs with AUC 219.075 (**Fig. 5**).

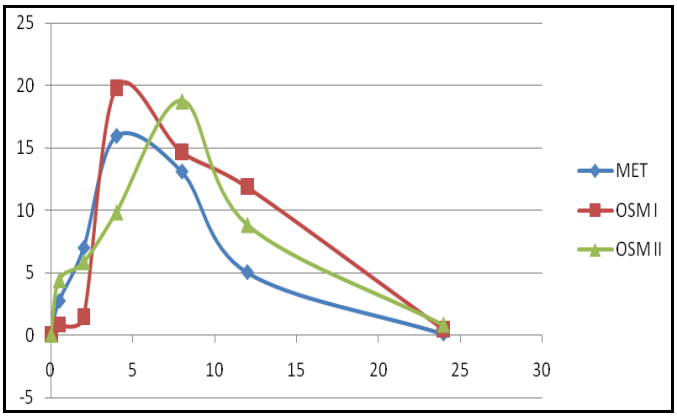

FIG. 5: COMPARISON OF MEAN PLASMA CONCENTRATION OF METFORMIN ALONE AND COMBINATION WITH EXTRACTS IN 24HRS.

II) Seven days protocol: It Showed the Cmax of 10.27ng/ml (**Table 10**).

While the combination of metformin with methanolic extract of *Ocimum sanctum*, show Cmax of 13.25ng/ml (**Fig. 6**).

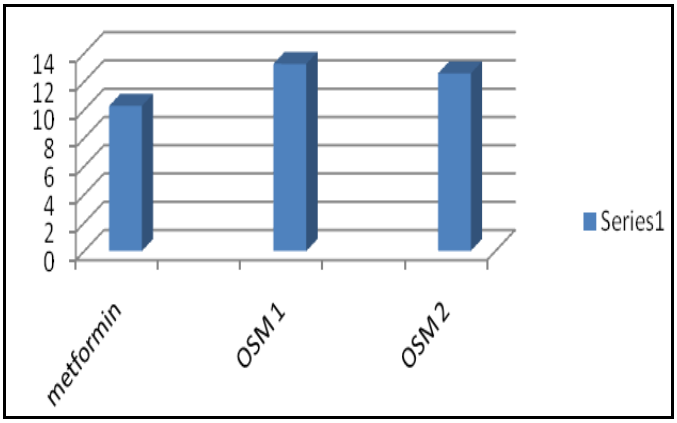

FIGURE 6: COMPARISON OF MEAN PLASMA CONCENTRATION OF METFORMIN ALONE AND COMBINATION WITH EXTRACTS ON 7TH DAY

OSM I- Ocimum sanctum 100mg/kg + Metformin 100mg/kg
OSM II- Ocimum sanctum 150mg/kg + Metformin 100mg/kg
(The values are expressed as mean, n = 6)

TABLE 10: PHARMACOKINETIC PARAMETERS (METFORMIN) ON 7 DAY STUDY

Sr No	Parameters	Results		
		Metformin100mg/kg)	Metformin(100mg/kg)+ *Ocimum sanctum* 100mg/kg	Metformin(100mg/kg)+ *Ocimum sanctum* 150mg/kg
1	Cmax (ng/ml)	10.27±0.616	13.25±0.392	12.57±0.658

4. DISCUSSION:

4.1 Drug:

A. The presence of Phytoconstituents mainly Alkaloids, Carbohydrates, Glycosides and Tannins.

B. The % yield of Methanolic extract of *Ocimum sanctum* was found to be 3.81% w/w.

C. Thin layer chromatography showed presence of chemical constituents like Eugenol, caryophylline and essential oils few of which may be responsible for enhancing activity of *Ocimum sanctum.*

4.2 Method:

HPLC method was developed and validated according to currently accepted US FDA guidelines of Bioanalytical method validation. The following parameters were tested.

A. Linearity

Metformin linearity was established in the range of 37.5ng/ml to 4800ng/ml. Correlation coefficients (r^2) were greater than 0.99 which meets the acceptance criteria.

Hence, the method was found to be linear.

B. Precision and Accuracy:

***a)* Intra-day (within batch) Precision and Accuracy:**

For LQC, MQC, & HQC, the intra-day precision was 4.27%, 14.41% and 16.06%, and the intra-day accuracy 98.80%, 111.92% and 107.60%, respectively. The results were found to be within the acceptance criteria.

***b)* Inter-Day (between batch) Precision and Accuracy:**

For LQC, MQC & HQC, the inter-day precision was 6.38%, 0.83% and 23.25%, and the interday accuracy 114.72%, 118.03% and 88.43%., respectively. The results found to be within the acceptance criteria.

Based on the intraday and inter day precision and accuracy studies, method was found to be precise and accurate.

C. Selectivity:
Selectivity is expressed in terms of % interference. The results were within the acceptance criteria and the response of interference peak at retention time of Metformin was found to be 0%. Hence, this method developed was found to be selective.

D. Sensitivity:
Sensitivity is measured in terms of LLOQ. The lowest limit of reliable quantification for Metformin was set at the concentration of the LLOQ, 37.5ng/ml.

The precision (% CV) and accuracy (% nominal) for Metformin at this concentration was found to be 1.09% and 99.86%, respectively. The results were within the acceptance criteria.

E. Stability Evaluation:
The Stability of Metformin was evaluated for stability *i.e.* Bench top stability in rat plasma of spiked plasma sample was evaluated.

The Metformin spiked plasma samples was found to be stable during above mentioned stability study.

4.3 Pharmacokinetic Study:
Protocol I: (24 hrs.)
I) After, oral administration of Metformin 100mg/kg a peak concentration of 15.96ng/ml (Cmax) was reached after 4 hrs. (Tmax). The half-life was found to be (2.23 hr.) Area under curve (AUC) was found to be 156.09.

II) After, oral administration of Metformin 100mg/kg and *Ocimum sanctum* 100mg/kg a peak concentration of 19.8ng/ml (Cmax) was reached after 3.027 hrs. (Tmax). The half-life was found to be (2 hr.) Area under curve (AUC) was found to be 219.075

III) After, oral administration of Metformin 100mg/kg and *Ocimum sanctum* 150mg/kg a peak concentration of 18.76ng/ml (Cmax) was reached after 8 hrs. (Tmax). The half-life was found to be (3.51 hr.) Area under curve (AUC) was found to be 194.66

Above observation indicates that the Cmax of Metformin is highest when given in combination with *Ocimum sanctum* 100mg/kg, the Tmax is also shifted to lower side, indicating that the plasma concentration maxima is attained earlier as compared to Metformin alone.

Protocol II: (7 days)
I) After, oral administration of Metformin 100mg/kg a peak concentration of 10.27ng/ml (Cmax)

II) After, oral administration of Metformin 100mg/kg and *Ocimum sanctum* 100mg/kg a peak concentration of 13.25ng/ml (Cmax)

III) After, oral administration of Metformin 100mg/kg and *Ocimum sanctum* 150mg/kg a peak concentration of 12.57ng/ml (Cmax).

Above observation indicates that the Cmax of Metformin is highest when given in combination with *Ocimum sanctum* 100mg/kg and 150mg/kg, as compared to Metformin alone.

CONCLUSION: The present study indicates that the methanolic extract of *Ocimum sanctum* may have antihyperglycemic activity. It also improves availability of metformin. Thus dose or frequency of dosing of metformin can be minimized and *Ocimum sanctum* may act as bioenhancer for metformin when administered orally.

REFERENCES:

1. Ohadoma SC, Michael HU. Effects of co-administration of methanol leaf extracts of *Catharanthus roseus* on the hypoglycemic activity of metformin and glibenclamide in rats. APJTM. 2001: 475-7.
2. Bailey JC. Traditional plant medicines as treatments for Diabetes. Diabetes care.1989; 12: 553- 64.
3. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. Journal of Ethnopharmacology. 2002; 81: 81-100.
4. Indian Pharmacopoeia, Vol. II, Government of India, Ministry of Health and Family Welfare, Published by the Indian Pharmacopoeia Commission, Ghaziabad. 2007; 740-741.
5. Thamma NK, Kota CM, Sreenivasulu R, Dr. Raju NSV, Reddy V. Novel RP-HPLC Method for the Estimation of Metformin Hydrochloride in Pharmaceutical Dosage forms. International Journal of Science Innovations and Discoveries.2011; 1(3): 395-421
6. Wanjari MM, There AW, Tajne MR, Umathe SN. Rapid and Simple RP-HPLC Method for the Estimation of Metformin in Rat Plasma. Indian J Pharm Sci. 2008; 70(2): 198–202.
7. Shah VP, Bioanalytical Method Validation—A Revisit with a Decade of Progress Pharmaceutical Research. 2000; 17(12): 1551-7.
8. Tiwari G, Tiwari R. Bioanalytical method validation: An updated Review. Pharmaceutical Methods. 2010; 2(1): 25-38.

9. Joshi A, Sharma V, Dubey BK. Development of an Analytical method to determine Eugenol Concentrations in alcoholic extracts of different Species of *Ocimum's*. International Journal of Phytopharmacy. 2011; 1 (2): 35-42.
10. Bioanalytical method Development and validation: Guidelines Available fromhttp://www.fda.gov/Drugs/ Guidance Compliance Regulatory Information /Guidances /default.
11. Islam MA, Akhtar A, Khan RI. Oral Glucose Tolerance Test (OGTT) in normal control and glucose induced hyperglycemic rats with *Coccinia cordifolia* L. and *Catharanthus roseus* L. Pak. Journal of Pharmaceutical Science. 2009; 22(4): 402-4
12. Eshrat H, Hussain MA, Kaiser J and Rao M. Hypoglycemic, Hypolipidimic and Antioxidant properties of Tulsi (*Ocimum Sanctum*) on Streptozotocin induced Diabetes in rats. Indian Journal of Clinical Biochemistry. 2001; 16(2): 190-4.
13. Rodrigues M, Alves G, Abrantes J. Drug Interaction of Fucus Vesiculosus Extract and Amiodarone in rats. Food and Chemical Toxicology. 2013; 52: 121–8.
14. Wagner H and Wolff P. New natural products and plant drugs with pharmacological, Biological and therapeutical activity, springer Verlag, Berlin.1-22.

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