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VIEWS

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Bioequivalence of Two Brivudine 125MG Formulations in Healthy Volunteers Under Fasting Conditions-A Pilot Study

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ABSTRACT

Brivudine is a nucleoside analogue used in the treatment of herpes zoster infections. The present study was aimed at analyzing the bioequivalence of the test product Brivudine 125mg with the reference product (Zostex (Brivudine) 125mg tablets of Menarini Group). The design was an open, longitudinal, randomized, comparative study of two formulations in single dose of 100 mg, with a 7 days washout in between doses. The study was an open label, randomized, two period, cross over, comparative study of the two formulations in single dose of 125mg. The study was conducted in 10 healthy adult male volunteers under fasting conditions with a wash out period of 7 days. Blood samples were collected post dose up to 72 hrs for pharmacokinetic analysis and safety evaluation was done by assessing the adverse events and laboratory tests. A validated LC-MS/MS method was used to determine the plasma concentrations of Brivudine. Bioequivalence between the products was established by calculating 90% confidence intervals (90% CI) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products. The 90% confidence intervals found for the relation Test/Reference, were C_{max} 56.22% to 118.5% and AUC_{0-t} 80.48% to 105.22%. According to European and FDA´s guidelines for Bioequivalence research, the confidence intervals for AUC_{0-t} ranged between 80.00-125.00%, however for C_{max} the lower limit was much lower indicating that the test and reference formulations of Brivudine were not bioequivalent.

Key Words: Brivudine, bioequivalence, pilot study.

Introduction

Herpes zoster is a cutaneous viral infection caused by the reactivation of varicella-zoster virus (VZV) from its latent state in the dorsal root ganglia several years after the initial exposure to the virus in the form of varicella (chickenpox) [1]. It usually manifests as a self-limited dermatomal rash with pain, but in some instances can cause complications, the most common of which is the postherpetic neuralgia (PHN) which is responsible for a significant economic burden. [2]

When administered in the first 72 hours, the systemic treatment of herpes zoster quickens the healing process, alleviates pain and also prevents other acute or chronic complications. This is mainly indicated in patients over the age of 50 and also in patients with head and neck involvement such as herpes zoster ophthalmicus. The drugs approved in Europe for the systemic treatment of herpes zoster are aciclovir, valaciclovir, famciclovir and brivudine.[3]

Brivudine is a nucleoside analogue which gets phosphorylated by viral thymidine kinase to form the active compound brivudine 5'-triphosphate. Brivudine 5'-triphosphate gets incorporated into the viral DNA and blocks the action of DNA polymerases, thus inhibiting viral replication. In clinical trials, Brivudine showed greater effectiveness against the varicella-zoster virus when compared to aciclovir and its derivatives, and also has the advantage of once a day dosing compared to multiple doses of the latter.[4]

Brivudine is rapidly absorbed after oral administration and undergoes extensive first pass metabolism, leading to 30% bioavailability. Maximum plasma concentrations are reached after 1 hr. Brivudine is highly (>95%) bound to plasma proteins and the terminal half-life is 16 hours. Brivudine has a very large distribution volume (75 L), suggesting that the drug is widely distributed in the tissues. [5]

Materials nd Methods

Volunteers

A total of 10 healthy adult human male volunteers between 20 to 45 years (both years inclusive) with BMI of 18.50 – 30.00 Kg/m^2 who were non smokers and non

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alcoholics were enrolled. Healthy volunteers as evaluated by medical history, vitals and general clinical examination, with normal or clinically insignificant biochemical, hematological, urinary, serology, Chest X Ray and ECG were selected.

Volunteers with history of any clinically significant cardiac, gastrointestinal, respiratory, hepatic, renal, endocrine, neurological, metabolic, psychiatric, hematological and/ or any major surgical procedure in the past three months were excluded from the study. Volunteers with history of alcoholism/ smoking/drug of abuse/ Hypersensitivity, present or past history of intake of drugs or any prescription drug or over the counter (OTC) drugs within 7 days which potentially modify kinetics / dynamics of Brivudine were also excluded from the study. Volunteers who consumed grapefruit and/or its products within 10 days prior to the start of study and subjects who had participated in any other clinical study or who had bled during the last 3 months were excluded from the study.

Informed Consent

The protocol and informed consent forms (ICFs) were reviewed and approved by an independent ethics committee prior to study initiation. All the volunteers were informed about the purpose, study nature, procedure, duration, anticipated risks and discomfort of the study in the vernacular language that they understand. A written informed consent was obtained from each one of them prior to study initiation and this clinical trial was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and national regulatory requirements [6, 7].

Study design

An open label, randomized, two treatment, two sequence, two period, single dose, cross over, bioequivalence study of Brivudine 125mg tablets and Zostex (Brivudine) 125mg tablets of Menarini Group in healthy, adult, human subjects under fasting conditions.

Study subjects received either test or reference in each period as per the randomization schedule. The randomization schedule was generated by using SAS® and each study subject was randomly assigned to one of the dosing sequences. On the day of check in at least 12 h prior to each dosing, all volunteers were screened for cocaine, cannabinoids, benzodiazepines, opioids, amphetamines, barbiturates and alcohol.

Drug administration

A single oral dose of test (T) or reference (R) of Brivudine was administered to study subjects in sitting posture at fixed time points with 240 ± 02 ml of water, at ambient temperature in each period as per randomization schedule. The subjects were fasted for 10 h prior to the dosing and no food was permitted until 4 h after dosing. Drinking water was not permitted 01 hour before dosing and until 01 hour post-dose, at all other times drinking water was permitted *ad libitum*. After administration of the oral dose, a mouth check was performed under supervision of quality control personnel to assess the compliance to this procedure. After dosing, the volunteers were to remain ambulatory or seated upright for the first 2 h. The subjects received standard food approximately at 04.00, 08.00 and 12.00 hours post-dose with time flexibility of +15 minutes. Wash out period of 7 days was given between two dosing periods.

Blood sampling

A total of 29 blood samples (3 ml each) were collected using pre-labeled vacutainers containing K3EDTA anticoagulant at -00.00 (Pre-dose), 00.25, 00.50, 00.75, 01.00, 01.33, 01.67, 02.00, 02.33, 02.67, 03.00, 03.33, 03.67, 04.00, 04.50, 05.00, 05.50, 06.00, 06.50, 07.00, 07.50, 08.00, 09.00, 10.00, 12.00, 16.00, 24.00, 48.00 and 72.00 hours post dose. First 23 samples were collected in the clinic through an indwelling cannula placed in a forearm / arm and remaining samples were collected as ambulatory samples through direct venous puncture.

Blood samples collected during the study were centrifuged at 4000 rpm for 10 minutes at 4 ± 2°C. Plasma was separated into single aliquot and stored at about -20°C or colder for analysis.

Analytical method

A validated LC-MS/MS bio-analytical method was used for estimation of Brivudine in plasma. Bioanalytical method validation was done as per FDA's Bioanalytical Method Validation guidance on Specificity, Sensitivity, Precision and Accuracy, Stability, Recovery and Dilution Integrity. Samples of subjects who completed the entire duration of study was analysed.

The method involved liquid/liquid extraction. The LC-MS-MS consisted of liquid chromatographic system (Agilent 1100, Germany), coupled with a triple quadrupole spectrometer (API 5000) from Applied Biosystems, (MDS Sciex, Canada), equipped with ESI source for the ionization (positive ionization mode). Integration was done using the Analyst 1.5.1 software (Applied Biosystems). Detection was done by multi reaction monitoring (MRM) mode, using the positive mode. The ion transition (m/z) for Brivudine was: 331.0/78.8. The ion transition for the internal standard was: 294/249.9.

The detector response for brivudine showed linearity over a concentration range of 10.118 to 3983.20 ng/ml (correlation coefficient ≥ 0.99). The lower limit of quantitation was 10.118 ng/ml for Brivudine.

Pharmacokinetic parameters and statistical analysis

The Pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ T_{max}, $T_{½}$, K_{el} and $AUC_{\%Extrap\_Obs}$) were calculated using Non compartmental Model of Phoenix® WinNonlin v

6.4. Statistical analysis was performed on the Ln-transformed pharmacokinetic parameters using SAS® v 9.2. The analysis included data from subjects who completed both the periods of the study.

The log-transformed pharmacokinetic parameters (C_{max} and AUC_{0-t}) were analyzed using ANOVA Model with the main effects of treatment, period, subjects nested within sequence and sequence as fixed effects. The log transformed primary PK parameters (C_{max} and AUC_{0-t}) were subjected to ratio analysis. The Test / Reference ratio was calculated for log transformed primary PK parameters.

To establish bioequivalence of the test product with that of reference product, 90% Confidence Interval (CI) for the ratio (Test/Reference) of Least Square Means of the natural log transformed PK parameters (C_{max} and AUC_{0-t}) must fall between 80.00% to 125.00%.

Results

Pharmacokinetics and statistics

In the present study, 10 healthy male volunteers who completed both the periods were included in the pharmacokinetic analysis.

The plasma concentration vs. time curve of test Brivudine 125 mg and reference Zostex 125 mg in fasting conditions is presented in Figure 1. The Geometric mean ratios, 90% CI, power and intra-subject coefficient of variation of test and references for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for are presented in Table1.The statistical results of primary pharmacokinetic parameters of Brivudine are presented in Table 2.

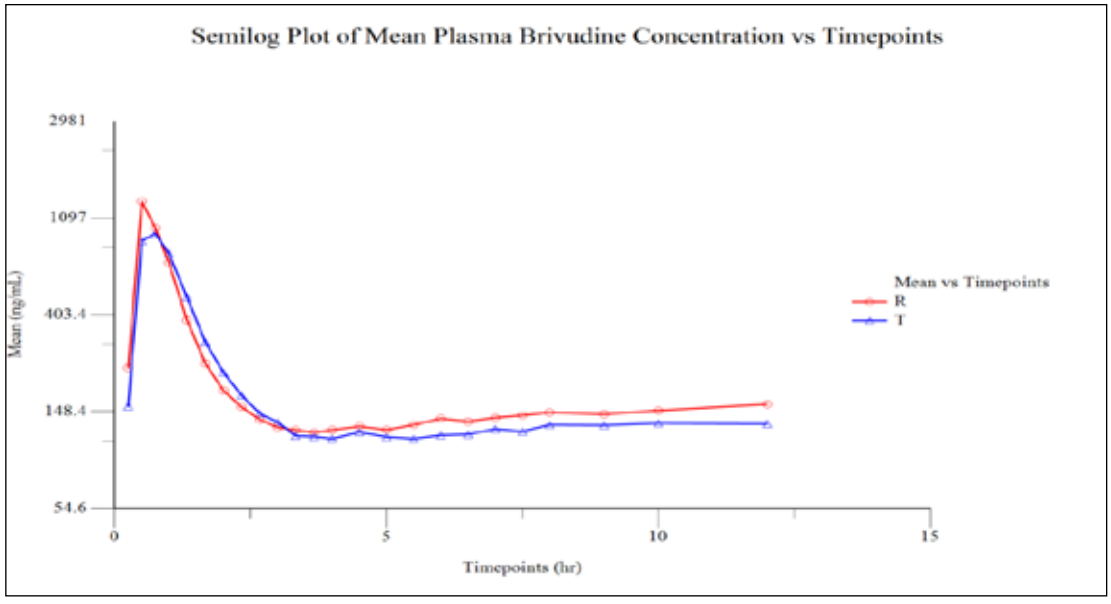

Figure 1: Bioavailability curve (Mean plasma concentration vs. Time) of test Brivudine 125 mg and reference Zostex 125 mg in fasting conditions.

Table - 1

The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for Brivudine 125mg are presented.

Dependent	T/R Ratio	Reference Geometric Mean	Test Geometric Mean	90% Lower Confidence	90% Upper Confidence	ISCV	Power
Ln(C_{max})	81.62%	1210.0764	987.7154	56.22%	118.50%	31.00%	0.2202
Ln(AUC_{0-t})	92.02%	2402.1436	2210.4812	80.48%	105.22%	10.93%	0.8852

Table - 2
Statistical results of primary pharmacokinetic parameters of Brivudine

Phamacokinetic parameters	Test geometric mean	Reference geometric mean	Test / reference ratio	90% confidence interval for test vs reference	Power of ANOVA
LN_Cmax	987.7154	1210.0764	81.62%	(56.22% TO 118.5%)	0.2202
LN_AUCt	2210.4812	2402.1436	92.02%	(80.48% TO 105.22%)	0.8852

Safety

Among the 10 volunteers who completed the study, only one subject administered with the reference product had fever and headache and was ascertained to be possibly related to the study drug.

Discussion

Bioequivalence studies allow for the interchangeability of generic products versus reference products without repeating clinical trials in patients [8].To establish therapeutic equivalence, the two drug products should contain the same active ingredient(s), with the same dosage form, route of administration and should be identical in strength or concentration. Two drug products (of the same active ingredient) are considered bioequivalent when the rate and extent of biologic absorption of the active ingredients is essentially similar when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions [9]. The extent of absorption is indicated by the Area under the curve (AUC) , whereas C_{max} and T_{max} are considered estimators of the rate of absorption. According to U.S. Food and Drug Administration and European Medicines Agency bioequivalence between two drug products can only be assumed when the characteristic parameters of bioavailability show no more than a defined difference, which depends on the nature of the drug, the subject population, and the clinical end point [10,11].

In this study, the pharmacokinetics of two Brivudine formulations were evaluated and compared in healthy male volunteers. The rate (Cmax) of absorption of the test formulation was significantly different from the rate of absorption of the reference formulation.

This being a pilot study, the ratios of LSM and 90% confidence intervals for the pharmacokinetic parameter (C_{max}) of Brivudine tablets 125 mg versus Zostex tablets 125 mg under fasting conditions were not within 80 to 125% FDA acceptance range for generic drug bioequivalence.

Conclusion

In this study, based on the statistical results, it can be concluded that the test and reference products did not satisfy the regulatory requirements of bioequivalence. The large intra subject variability of 31 % and T/R ratio of 81.62% obtained for Cmax in this study, indicates that more number of subjects and suitable study design needs to be incorporated in future pivotal trials. However with respect to adverse events, the test product was well tolerated with no significant adverse effects as compared to the reference product.

References

1. [Guideline] Dworkin RH, Johnson RW, Breuer J, Gnann JW, et al. Recommendations for the management of herpes zoster. *Clin Infect Dis*. 2007 Jan 1. 44 Suppl 1:S1-26

2. Blein C, Gavazzi G, Paccalin M, Baptiste C, Berrut G, Vainchtock A. Burden of herpes zoster: the direct and comorbidity costs of herpes zoster events in hospitalized patients over 50 years in France. *BMC Infect Dis*. 2015 Aug 19. 15 (1):350.

3. Espana A, Redondo P. Update in the treatment of herpes zoster. Actas dermo-sifiliograficas. 2006 Mar;97(2):103-14.

4. Espana A, Redondo P. Update in the treatment of herpes zoster. Actas dermo-sifiliograficas. 2006 Mar;97(2):103-14.

5. Rabasseda X. Brivudine: a herpes virostatic with rapid antiviral activity and once-daily dosing. Drugs today. 2003 May 1;39(5):359-71.

6. World Medical Association Declaration of Helsinki, Ethical principles for the medical research involving human volunteers adopted by the: 59th General Assembly Seoul, Korea, 2008

7. ICH Expert Working Group, Guideline for Good Clinical Practice E6 (R1): International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. J Postgrad Med ,1996, 47: 45-50.

8. Perry R, Perspectives on the bioequivalence and therapeutic equivalence of generic formulations: An

overview of the landscape. Clin Ther , 2010, 32:1796-1797.

9. Chen ML, Shah V, Patnaik R, Adams W, Hussain A, Conner D, Mehta M, Malinowski H, Lazor J, Huang SM, Hare D. Bioavailability and bioequivalence: an FDA regulatory overview. Pharmaceutical research. 2001 Dec 1;18(12):1645-50.

10. Food and Drugs Administration,Guidance for industry Statistical Approaches to Establishing Bioequivalence, 2009.

11. The European Agency for the Evaluation of Medicinal Products EMA, Committee for Proprietary Medicinal Products (CPMP) Note for guidance on the investigation of Bioavailability and Bioequivalence, London, 2001.

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SOLUBILITY ENHANCEMENT OF ASENAPINE WITH POLOXAMER188 BY SOLVENT EVAPORATION METHOD

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ABSTRACT

Asenapine (AS), second-generation (atypical) antipsychotic drug is characterized by low solubility in gastric fluid and low dissolution rate. Its oral bioavailability is less than 2% due to poor solubility and first pass metabolism. The aim of the present investigation was to enhance the aqueous solubility and therapeutic efficacy of the drug by formulating solid dispersions (SD) of AS with a hydrophilic carrier Poloxamer 188(PXM) by solvent evaporation method. Phase solubility study with increasing PXM concentrations (0.5 to 2 % w/v) was done to study the influence of polymer concentration on solubility of AS. SD's of AS and PXM in 1:1, 1:3 and 1:5 w/w ratios were prepared by physical mixing and solvent evaporation method, followed by dissolution studies. Evaluation of the properties of the SDs was performed by using dissolution, Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies. The SDs of AS with PXM exhibited more enhanced dissolution rate than physical mixture and pure drug, and the rate increased with increasing concentration of Poloxamer 188 in SDs. The FTIR spectroscopic studies showed the stability of AS and absence of well-defined AS – PXM interaction. The DSC and XRD studies indicated that the transformation of Asenapine from crystalline to amorphous state by solvent evaporation method.

Keywords: Asenapine, Poloxamer 188, solvent evaporation, solubility

Introduction

Poorly water soluble BCS class II entities, having high permeability, affiliated with obtuse drug absorption which leads to scarce and varying bioavailability. It assumes that the rate of absorption is depends on the drug solubility and dissolution, successive transport on intestinal membrane and liver. Attempts to improve the solubility of these drug candidates have been performed by various approaches [1]. Among them, solid dispersion technique has attracted considerable interest as an efficient means of improving the dissolution rate, which increases the bioavailability of a range of poorly aqueous soluble drugs [2–4]. Fast and immediate drug dissolution from solid dispersions has been observed due to increased wettability, improved dispersibility of drug particles, and existence of the drug in amorphous form with improved solubility and absence of aggregation of drug particles using various hydrophilic carriers [5–6].

Asenapine is a BCS class II drug, used for the treatment of schizophrenia and bipolar mania [7]. Exact mechanism of Asenapine and other antipsychotic agents in schizophrenia and bipolar disorder unknown; efficacy in schizophrenia may be mediated through a combination of antagonist activity at central dopamine type 2 (D2) and serotonin type 2 (5-hydroxytryptamine [5-HT2A]) receptors. Asenapine (ASP) undergoes extensive first pass metabolism, with oral bioavailability only 2%. The present research work deals with the study of Asenapine solubility enhancement by using solvent evaporation technique [8].

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MATERIAL AND METHODS

Materials

Asenapine was gifted by Dr. Reddy's Laboratories Ltd., Hyderabad, poloxamer 188 purchased from S.D. Fine Chemicals Ltd. All other chemicals used were of analytical grade and procured from commercial sources.

Preparation of physical mixtures

Physical mixtures were prepared by mixing of Asenapine and poloxamer 188 in mortar and pestle according to 1:1, 1:3, 1:5 ratios by geometrical dilution method. The geometric mix blends passed through sieve no#60 and kept in the desiccator [12].

Preparation of SD by solvent evaporation technique

Asenapine and poloxamer 188 were weighed according to 1:1, 1:3, 1:5 ratios. Approximately 20 ml of methanol was taken as common solvent to solubilise drug and polymer in different ratios forming a clear solution. Then the solution poured into petridish, kept overnight for evaporation of solvent at room temperature. Then the resultant product was scrapped away kept in the desiccator for 24hrs to complete removal of moisture and passed through sieve no#60 and kept in the desiccator [14-16].

Phase solubility analysis

The solubility of Asenapine was determined in water and pH 6.8 phosphate buffer medium. The effect of concentrations of Poloxamer 188 on the equilibration solubility of Asenapine in water and pH 6.8 phosphate buffer medium at room temperature was carried out by adding an excess quantity of drug (20 mg) into a screw-capped glass vial containing 20 ml of solvent with various concentrations of the carrier. The suspension were shaken for 24hrs on a rotary bath shaker & filtered through Whatman no.1 filter paper. The filtrate so obtained was diluted & analyzed spectrophotometrically [9-11].

Analysis of drug content in solid dispersions

The drug content of Asenapine in each physical mixtures and solid dispersions were determined using UV-spectroscopy. Accurately weighed quantity of solid dispersion or physical mixture equivalent to 10 mg of Asenapine was transferred to 100 ml of volumetric flask and volume was made up to 100 ml with methanol and 1 ml of this solution was taken and it was diluted to 10 ml with methanol and absorbance was noted at 270 nm, concentration of Asenapine was determined using calibration curve of Asenapine in methanol.

Percentage yield value

The Percentage yield value of solid dispersions and physical mixtures were measured by the following formula.

Percent yield value= (Practical yield value / Theoretical yield value) X 100

Characterization of Solid Dispersion

Fourier transform infrared spectroscopy (FT-IR):

The FT-IR spectra were obtained using FT-IR spectrometer (Shimadzu).The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix in 1:5 (sample : KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Forty five scans were obtained at a resolution of 4 cm^{-1} from 4500 to 400 cm^{-1}.

Differential Scanning Calorimetry:

The DSC measurements were performed on a Pyris Diamond TG/DTA differential scanning calorimeter with thermal analyzer. All accurately weighed samples (about 5 mg) were placed in sealed aluminum pans. An empty aluminum pan was used as reference.

X-ray diffraction

The X-ray powder diffraction patterns were obtained by using Philips Holland PW 1710 with Cu Kα (λ = 1.54056A°) radiation and a crystal monochromator, voltage: 45 mv and current: 20 amps. The diffraction patterns were run at 2^0/min in terms of 2θ angle.

In-*vitro* Dissolution rate studies

The in vitro dissolution studies of physical mixtures and solid dispersions of Asenapine were carried out on USP type II dissolution apparatus and the results were compared with those for pure Asenapine. The dissolution vessels contained 900 mL of phosphate buffer pH 6.8 maintained at 37 °C ± 0.5 °C and paddle speed set at 50 rpm. Solid dispersions equivalent to 20 mg of Asenapine were added to the dissolution medium in a powder form. Then, 5 mL samples were withdrawn at 5, 10, 20, 30, 45 and 60 min from the dissolution medium. The withdrawn sample was replenished with 5 mL of fresh media. The withdrawn samples were analyzed for Asenapine content by measuring the absorbance at 270 nm using UV-visible spectrophotometer (Shimadzu). Dissolution studies for each formulation were performed in triplicates.

Results and Discussion

Phase solubility study

The phase solubility curves of pure Asenapine in the presence of Poloxamer 188 at 25 ºC are shown in Figure-1. The apparent solubility of Asenapine increased with increasing carrier concentrations. Using the highest carrier concentration, the solubility increased approximately 2.13 fold in distilled water and 2.44 fold in pH 6.8 phosphate buffer as compared to pure drug. The solubility found in this study for Asenapine at 25 ºC was 2.115 mg/mL in distilled water and 2.358 mg/mL pH 6.8 phosphate buffer.

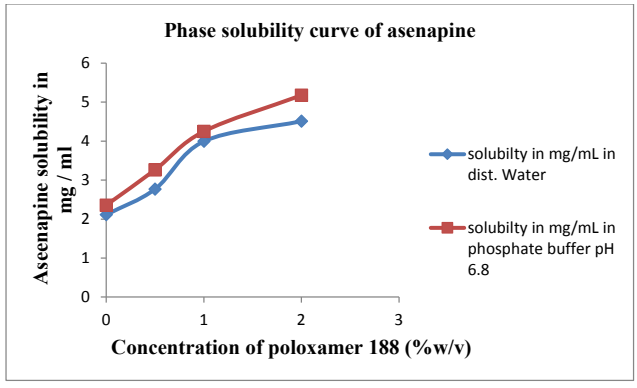

Fig.1: Phase solubility diagram of Asenapine/ poloxamer 188 system in water/ pH 6.8 phosphate buffer.

Percent Yield and Drug Content

The percent yield of various Asenapine physical mixtures and solid dispersions was within the range of 92.09 ± 0.16% to 99.18 ± 0.29% (Table 1). The percentage drug content in physical mixtures and solid dispersions was within the range of 95.09 ± 0.12% to 99.24 ± 0.29% and 90.75 ± 0.91% to 94.60 ± 0.13% respectively as reported in Table 1. This indicated that drug was uniformly distributed in all of these prepared physical mixtures and solid dispersions.

Characterization of solid dispersion

In-*vitro* Dissolution rate studies

The in vitro dissolution profiles of the drug, various solid dispersions using poloxamer 188 and their respective physical mixtures in phosphate buffer (pH = 6.8) are shown in Figures 2 and 3. All of the physical mixture and solid dispersion samples showed improved dissolution of Asenapine. The enhancement of dissolution is mainly attributed to increased surface area of drug exposed to large carrier molecules and increased wettability. Again, all of the solid dispersion samples showed more improved Asenapine dissolution than their respective physical mixture samples. This observation indicated that the increased dissolution of Asenapine from solid dispersion due to presence of drug in amorphous state as compared to the physical mixtures and pure drug, where drug is present in crystalline state.

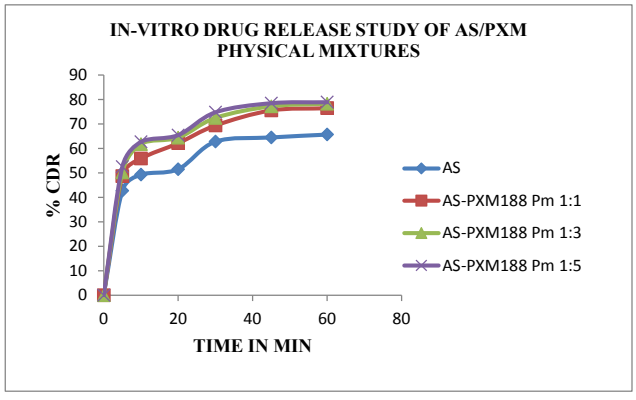

Fig.2: In-vitro drug release study of Asenapine and poloxamer 188 physical mixtures

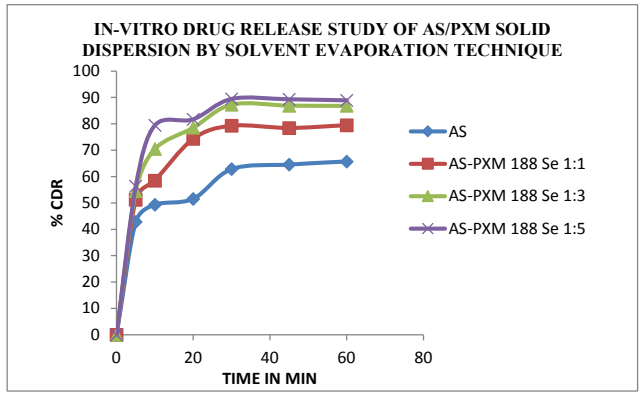

Fig.3: In-vitro drug release study of Asenapine and poloxamer 188 solid dispersions by solvent evaporation technique

FTIR Spectroscopy Analysis

FTIR spectroscopy analysis was done to analyze physicochemical interactions between Asenapine and poloxamer 188. FTIR spectra of pure Asenapine, poloxamer 188 and physical mixture are shown in Figure 4. The characteristic peaks of pure Asenapine were found to be present in the spectra of physical mixture. This finding reveals the lack of interaction between the drug and the carrier in the sample.

DSC Analysis

DSC analysis was done for pure Asenapine and solid dispersions using poloxamer 188 are shown in Figure 5. The DSC thermogram of pure Asenapine showed a sharp endothermic peak at 143.46◦C, corresponding to its melting point. The DSC curve of poloxamer 188 showed a sharp peak endothermic peak at 64.1◦C. The DSC curve of solid dispersion showed reduction of melting point to 142.6 deg Centigrade range with widening of peak. This reduction in melting point and broadening of peak was an indication of conversion of crystalline fraction into amorphous one. We can assume a positive conversion here.

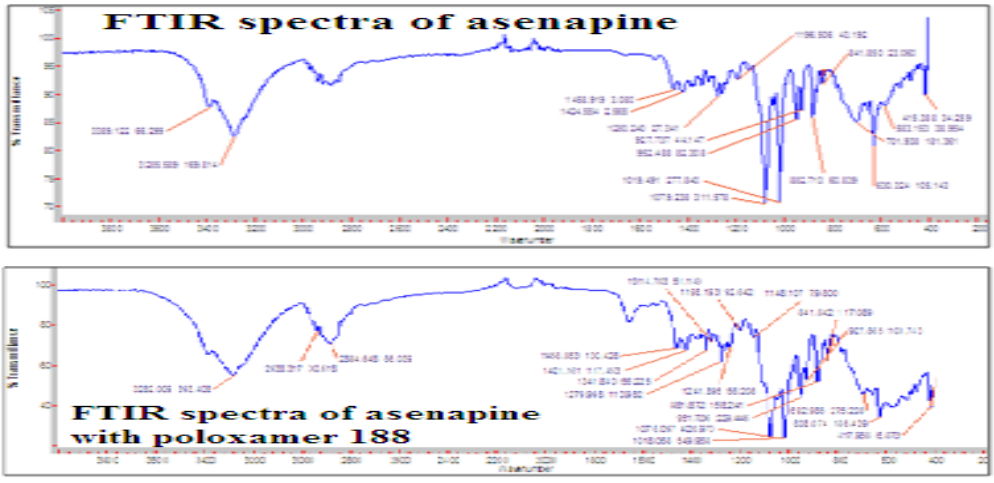

Fig.4: FTIR spectra of pure Asenapine and its physical mixtures with poloxamer 188.

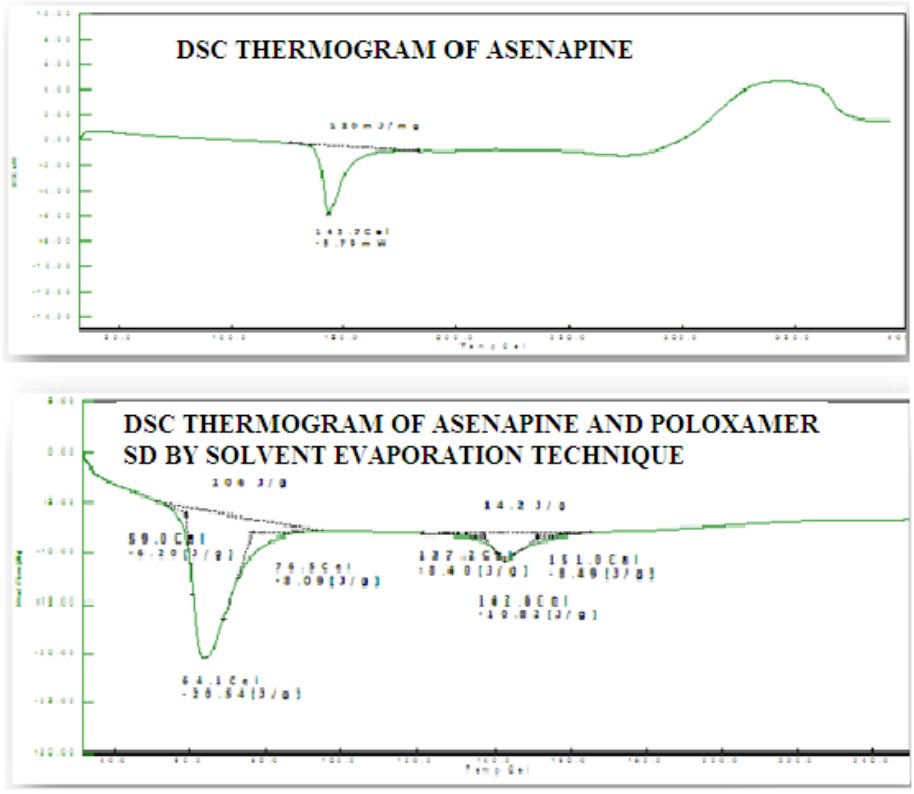

Fig.5: DSC thermogram of Asenapine and its solid dispersion with poloxamer 188 prepared by solvent evaporation technique

X-ray diffraction

X-ray diffraction spectra of pure Asenapine and solid dispersion are illustrated in Figure 6. The presence of sharp distinct peaks in Asenapine spectra indicated its high crystallinity. The diffraction spectrum of Asenapine showed that the drug was of crystalline in nature as demonstrated by numerous, distinct peaks at 2θ of 10.4, 14.2, 15.4, 20.1, 22.9, 25.1 and 27.2. The spectrum solid dispersion prepared with poloxamer 188 showed a reduction in the total number of peaks, base broadening of appeared peak along with a reduction in peak intensity providing convincing evidence for the formation of amorphous form in solid dispersion. The result indicated that the drug in solid dispersion was in amorphous form. Hence, increased dissolution of the drug was observed.

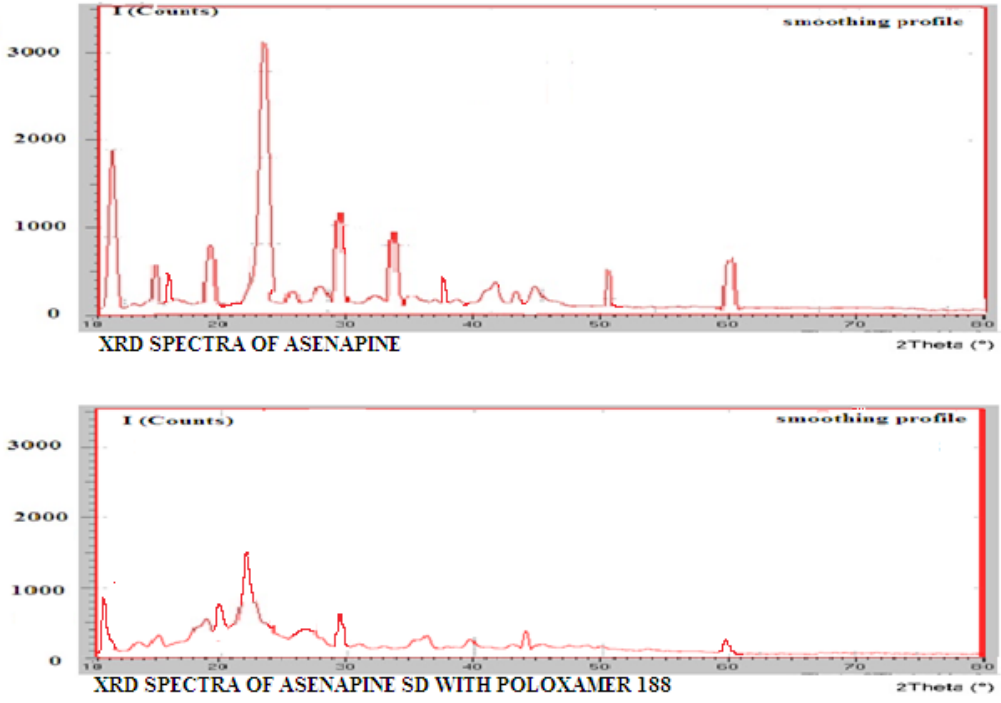

Fig.6 XRD data of Asenapine and its solid dispersion with poloxamer 188 prepared by solvent evaporation technique

CONCLUSION

In this work, solid dispersions were prepared with Asenapine and poloxamer 188 by solvent evaporation technique. In the phase solubility study, the drug showed better solubility in phosphate buffer pH 6.8 than distilled water. The apparent solubility of Asenapine increased with increasing carrier concentrations. Solid dispersions showed better dissolution of Asenapine than physical mixtures. Solid dispersion of AS: PXM 188 Se (1:5) showed the maximum dissolution efficiency among all solid dispersions and physical mixtures. IR spectra indicated no well-defined interaction between the drug and polymer. DSC thermograms of solid dispersion indicated complete miscibility of the drug in carrier. Amorphous nature of the drug in solid dispersion was confirmed by a decrease in enthalpy of drug melting in solid dispersion compared to the pure drug. XRD analysis indicated a reduction in drug crystalline nature in solid dispersion. In conclusion, these results could be an indication that solid dispersion prepared by the solvent evaporation method could be useful for the development of pharmaceutical products containing Asenapine.

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REFERENCES

1. Kumar BP, Rao S, Murthy KVR, Sahu RK, Ramu B. Solid dispersion technique: A tool for enhancing bioavailability of poorly soluble drugs. Journal of Chemical and Pharmaceutical Sciences 2011; 4(4): 170- 179.
2. Verma S, Rawat A, Kaul M, Saini S. Solid dispersion: A strategy for solubility enhancement. International Journal of Pharmacy and Technology 2011; 3(2): 1062-1099.
3. Patidar K, Kshirsagar MD, Saini V, Joshi PB, Soni M. Solid Dispersion Technology: A Boon for Poor Water Soluble Drugs. Indian Journal of Novel Drug delivery 2011; 3(2): 83-90.
4. Kumar S, Gupta SK, Sharma PK. Dissolution Rate Enhancement of Aceclofenac by Solid Dispersion Technique. Asian Journal of Pharmacy and Life Science 2011; 1 (4): 396-400.
5. Kulkarni PK, Dixit M, Panner S, Jain Achin. Preparation and Evaluation of Naproxen solid Dispersion Technique. International Research Journal of Pharmacy 2012; 3(9): 174-177.
6. Dua K, Pabreja K, Ramana MV. Preparation, Characterization and In Vitro Evaluation of Aceclofenac Solid Dispersions. ARS Pharm 2010; 51 (1): 57-76.
7. www.drugs.com/ Asenapine
8. www.formulationdiary.com/ Asenapine
9. Habeeba P, Madhavanb N, Gladisa K, Anithaa Y, Mohammedc S, Raghunathc P. Formulation, Optimization and Evaluation of Solid Dispersion Tablets of Aceclofenac Using Kollidon 30. International Journal of Biopharmaceutics 2013; 4(1): 10-17.
10. Panigrahi R, Behera SP,.Murthy PN. Enhancement of Solubility of Gliclazide by Solid Dispersion. International Journal of PharmTech Research 2011; 3(2): 1118-1124.
11. Ibrahim EH, El-Faham TH, Mohammed FA, El-Eraky NS. Enhancement of Solubility and Dissolution Rate of Domperidone by Utilizing Different Techniques. Bull. Pharm. Sci 2011; 34(2): 105-120.
12. Gupta S, Ranawat MS. Effect of Hydrophilic Polymers on Solubility Enhancement of Oral Antidiabetic Agent by Solid Dispersion Technique. Asian Journal of Biochemical and Pharmaceutical Research 2012; 2 (2): 299-306.
13. Rote H, Thakare VM, Tekade BW, Zope RP, Chaudhari RY, Patil VR. Solubility Enhancement of Glipizide Using Solid Dispersion Technique. World Journal of Pharmaceutical research 2012; 4(1):1096-1115.
14. Rao AB, Shivalingam MR, Reddy K, Rao S, Rajesh K, Sunitha N. Formulation and Evaluation of Aceclofenac Solid Dispersions for Dissolution Rate Enhancement. International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(2): 146-150.
15. Kumar VR, Sevukarajan M, Vulava J, Pavankumar AG, Deepthi Y, Manjunath M, Anand A. Improvement of Dissolution Characteristics and Bioavailability of Tadalafil by Solid Dispersion Technique Using Water-Soluble Polymers. International Journal of Advanced Pharmaceutics 2012; 2(2): 56- 63.

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In Vitro Antioxidant Activity of Hydroalcoholic Extract of *Momordica cymbalaria* fruits

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ABSTRACT

Antioxidants are vital substances which possess an important role to protect human beings against infections and degenerative diseases caused by free radical induced oxidative stress. The main aim of present study is to evaluate the *In vitro* antioxidant activity of hydroalcoholic extract of *Momordica cymbalaria* fruits. The antioxidant activity of *Momordica cymbalaria* fruits was assessed by different *In vitro* experiments including DPPH radical assay, Metal chelation assay, Reducing activity assay and Total antioxidant assay for Ascorbic acid equivalents, Total Phenolic content for Gallic acid equivalents and Total flavonoids content for Quercetin equivalent. *Momordica cymbalaria* fruits showed moderate free radical scavenging activity as evidenced by the IC_{50} values in DPPH (651.19 µg/ml), in Metal chelation (410.32 µg/ml) scavenging assays, total antioxidant activity (44.10±0.3 µg) and reducing power assay (20.00±0.09 µg) for ascorbic acid equivalent for mg of plant extract. Furthermore the Total phenolic content and Total flavonoids content were found to be 140.02±0.05 µg gallic acid equivalent per mg of extract and 32.00±0.17 µg for quercetin equivalent per mg of extract respectively. In conclusion, results of the present study demonstrated that Momordica cymbalaria fruits could be used as a viable source of natural antioxidants for development of various neutraceutical products.

Introduction

Free radicals are described as unstable, highly reactive chemical species and play an important role in human health and valuable in fighting against various pathological conditions such as tissue injury, inflammation process and neurodegenerative diseases. When its count of these free radicals exceed in the body, it can damage the cells and initiate the several disorders [1].

Antioxidants have cardinal role to protect the human body against the free radicals induced oxidative stress. Synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to mitigate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and liver [2]. Therefore, investigations of antioxidants are focused on naturally occurring substances, especially plant phytochemical constituents.

Many research reports are clearly confirmed that herbal plants, foods (vegetables, leafy greens and tea) are rich in antioxidants play an essential role in the prevention of free radicals induced disorder and has been revealed that various phenolic compounds such as flavonoids, tannins, coumarins, xanthones and procyanidins scavenge radicals dose-dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies [3, 4].

Momordica cymbalaria is one of the species of cucurbitaceae family. The plant is a perennial climber available only during the monsoon season and is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. The plant is a climbing annual or perennial herb with slender, scandent, branched, striate stem. Earlier reports were suggested that, it is proved as antidiabetic and hypoglycemic agent [5], hepatoprotective agent [6], Cardioprotective agent [7], antimicrobial agent [8], Nephroprotective agent [9], anti cancer agent [10] and anti implantation and anti ovulatory agent [11]

In view of our screening project, hydroalcoholic extract of *Momordica cymbalaria* fruits was investigated to assess their antioxidant properties in different antioxidant property determination assays include DPPH radical scavenging method, Metal chelation assay, Total antioxidant activity, Reducing activity assay, Total Phenol test and

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Total Flavonoids test studied in this report. Ascorbic acid, Gallic acid and Quercetin were used as antioxidant standard compounds respectively.

Material and methods

Collection and authentication of plant material

Plant fruits of were collected from the local market of Kadapa, Kadapa district, Andhra Pradesh, India and were authenticated by Dr. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal, Telangana State, India; Voucher specimen was stored in the department of Pharmacognosy, CMR college of Pharmacy, Hyderabad, Telangana state, India. The fruits were air-dried, ground to powder and stored in an airtight container.

Preparation of extract

The powder (100 g) was mixed with water-alcohol (30:70) in a round bottom flask and left seven days at room temperature with occasional stirring. Next day, the mixture was filtered with Whatman filter paper no.1. The filtrate was evaporated *in vaccuo* using rotary film evaporator. The extract was stored at 4 °C for further studies.

Chemicals

Chemicals such as 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Ferrozine were procured from Sigma Chemical Co. (INDIA). All other chemicals unless and otherwise mentioned were obtained from Hi-media Laboratories and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India).

Spectrophotometric Measurements

Spectrophotometric measurements were performed by UV-VIS Double Beam Spectrophotometer **(ELICO SL-210).**

In vitro antioxidant activity assays

DPPH radical scavenging ability was assessed according to the method of Blois, 1958 [12], Metal chelating ability was carried out according to Dinis et al., 1994 [13], Total antioxidant activity was eluted by using the method described by Prieto et al., 1999. [14], Reducing power assay was carried out according to the method of Manisha et al., 2009 [15], Total flavonoid content was determined according to modified method of Zhishen et al., 1999 [16] and total phenol content was determined according to the Folin- Ciocalteu method [17].

Statistical Analysis

The experimental results were expressed as mean ±standard deviation (SD) of three replicates.

Results and Discussion

DPPH radical scavenging activity

It is an extensively used, relatively rapid and accurate method for the assessment of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical nature and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually evident as a change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity [18]. *Momordica cymbalaria* was exhibited a comparable antioxidant activity with that of standard ascorbic acid. Figure: 01 showed DPPH radical scavenging activity of standard ascorbic acid. The IC_{50} values found to be 651.19 and 6.8 µg/ml for the plant extract and standard ascorbic acid respectively.

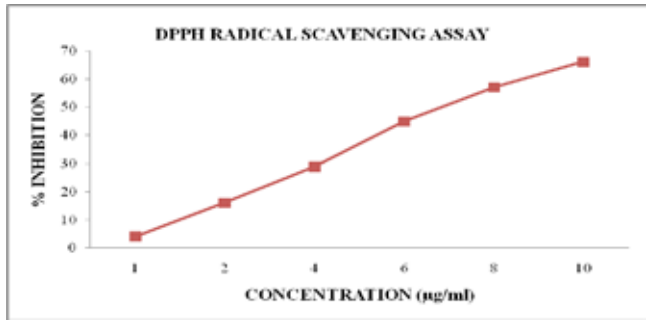

Fig.1: DPPH radical scavenging activity of standard Ascorbic acid.

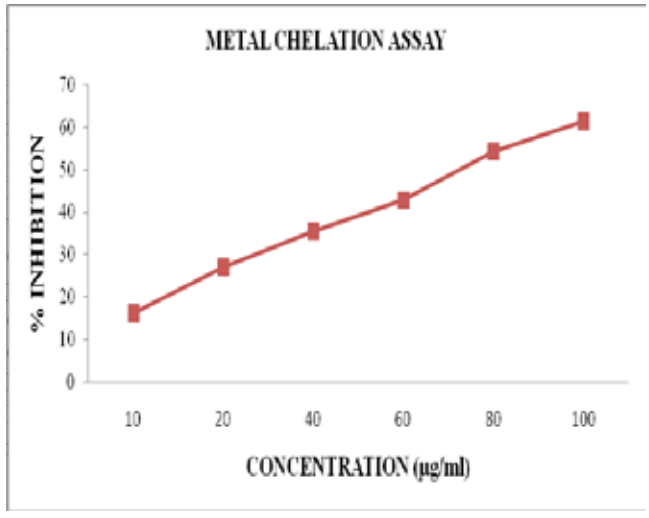

Fig. 2 : Metal chelation activity of standard EDTA.

Total antioxidant activity

The assay was based on the reduction of Mo(VI)-Mo(V) by the extracts and subsequent formation of a green phosphate/Mo(V) complex at acidic P^H [14]. Figure: 03 showed total antioxidant activity of standard ascorbic acid.

Total antioxidant activity of the hydroalcoholic extract of *Momordica cymbalaria* was found to be 44.10±0.3 µg ascorbic acid equivalents per mg of plant extract.

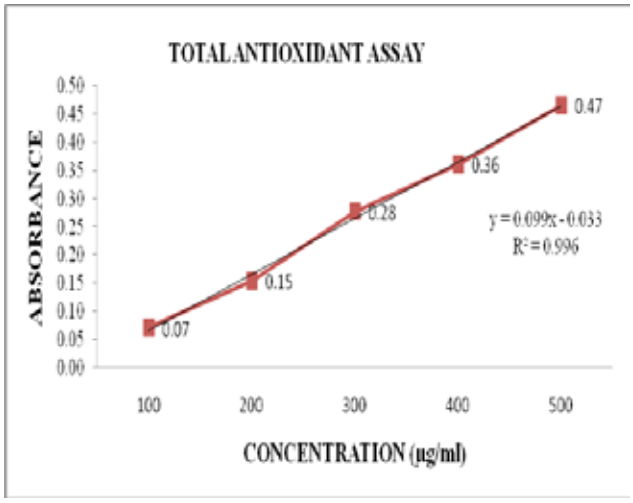

Fig. 3: Total Antioxidant Activity of standard Ascorbic acid.

Reducing power assay

In the present assay, the reducing ability of the plant extract was confirmed by transformation of Fe^{3+} to Fe^{2+} [15]. The reducing ability of a substance may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Figure: 04 showed reducing power assay of standard ascorbic acid. Reducing power ability of the hydroalcoholic extract of *Momordica cymbalaria* found to be 20.00±0.093 µg ascorbic acid equivalents per mg of plant extract.

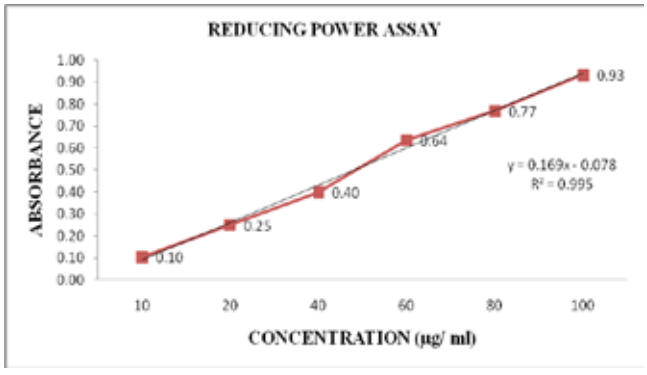

Fig. 4: Reducing Power Assay of standard Ascorbic acid.

Total flavonoid content

The principle of this method is that aluminum chloride forms acid stable complexes with C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones and flavonols [16]. In addition, aluminum chloride forms acid stable complex with ortho- di hydroxyl groups in the A or B rings of the flavonoids. Figure: 05 showed total flavonoid content of standard Quercetin. Total flavonoid content of the hydroalcoholic extract of *Momordica cymbalaria* was found to be 32.02±0.07 µg quercetin equivalent per mg of plant extract.

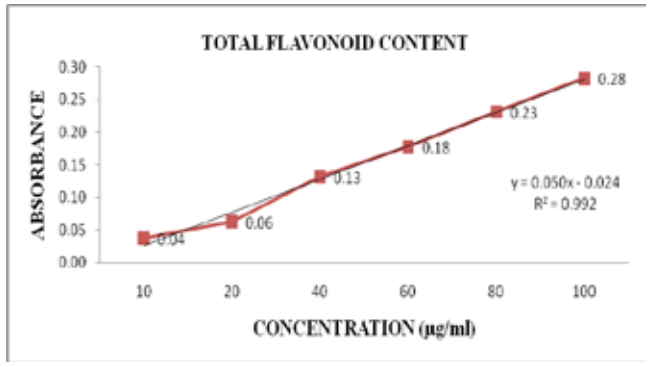

Fig. 5: Total Flavonoid Activity of standard Quercetin.

Total phenol content

The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [**17**].

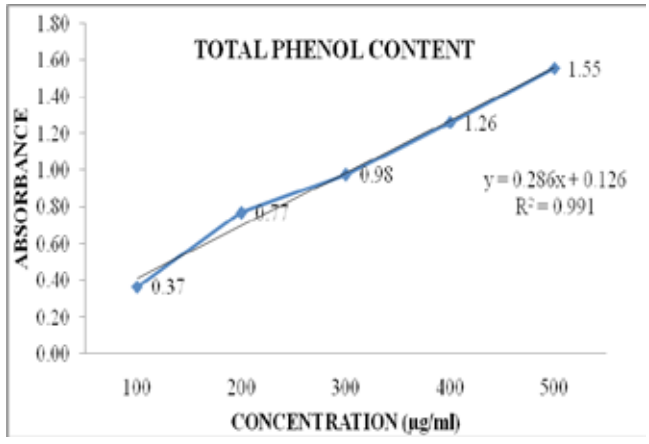

Fig. 6: Total Phenol Content of standard Gallic acid

Figure: 06 showed total phenol content of standard Gallic acid. Total phenol content of the hydroalcoholic extract of *Momordica cymbalaria* fruits was found to be 140.02±0.052 µg gallic acid per mg of plant extract.

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References

1) Nayan R. Bhalodia, Pankaj B. Nariya, Acharya RN, Shukla VJ. Evaluation of in vitro Antioxidant activity of Flowers of Cassia fistula Linn. Int J Pharm Tech Res 2011; 3(1): 588-599.

2) Zengin G, Aktumsek A, Gokalp Guler O, Yavuz Selim Cakmak YS, Evren Yildiztugay. Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz. Rec Nat Prod 2011; 5(2): 123-132.

3) Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, Toxicology Supplement 1998; 20: 237.

4) Azam S, Hadi N, Khan NU, Hadi SM. Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate:implications for anticancer properties. Toxicology in Vitro 2004; 18: 555.

5) Kameswara Rao B, Kesavulu MM, Giri R, Appa Rao CH. Antidiabetic and hypolipidemic effects of Momordica cymbalaria Hook. fruit powder in alloxan-diabetic rats. J Ethnopharmacol 1999 ; 67: 103-109.

6) Pramod Kumar, Deval Rao G, Lakshmayya, Ramachandra Setty S. Antioxidant and hepatoprotective activity of tubers of Momordica tuberosa Cogn. against CCl4 induced liver injury in rats. Indian J Exp Biol 2008; 46: 510-513.

7) Koneri R, Balaraman R, Vinoth KM, Hariprasad. Cardioprotective effect of Momordica cymbalaria fenzl against experimental myocardial injury induced by isoproterenol. Int J Pharmacol 2008; 5: 2.

8) Sangeetha sajjan SH, Chetana, Padmaa M Paarakh, Vedamurthy AB. Antimicrobial activity of Momordica cymbalaria Fenzl aerial parts extracts. Ind J Nat Prod & Resources 2010; 3: 296-300.

9) Pramod Kumar, Devala Rao G, Lakshmayya S. Ramachandra Setty S. Nephroprotective and nitric oxide scavenging activity of tubers of Momordica tuberosa in rats. Avicenna J Med Biotech 2011; 3(2): 87-93.

10) Jeevanantham P, Vincent S, Balasubramaniam A, Jayalakshmi B, Senthil Kumar BN. Anti cancer activity of methanolic extract of aerial parts of Momordica cymbalaria Hook F. against Ehrlich ascites carcinoma in mice. J Pharm Sci 2011; 3: 1408-1411.

11) Koneri Raju, Saraswati CD, Balaraman R, Ajeesha EA. Anti implantation activity of the ethanolic root extract of Momordica cymbalaria Fenzl in rats. Ind J Pharmacol 2007; 39: 90-96.

12) Blois, MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 29:1199-2000.

13) Dinis TCP, Madeira VMC, Almeidam LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radical's scavengers. Arch Biochem and Biophy 1994; 315: 161-169.

14) Prieto P, Pineda M, Aguilar M., Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999; 269: 337-341.

15) Pandey Manisha, Sonker Kanchan, Kanoujia Jovita, Koshy MK, Saraf Shubhini A. *Sida Veronicaefolia* as a Source of Natural Antioxidant IJPSDR 2009; 1: 180-182.

16) Zhishen J, Mengcheng T, Jianming W, The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64: 555-559.

17) Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. Am J Enol Viticult 1977; 28: 49–55.

18) Shazia Usmani. Screening for antioxidant and free radical scavenging potential of extracts of leaves and flowers of *Calotropis gigantea*. Asian J Pharm Clin Res 2013; 6: 97-100.

19) Chandra Mohan S, Balamurugan V, Thiripura Salini S, Rekha R. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata.* J Chem Pharm Res 2012; 4: 197-202.

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