ISSN 0973 – 9874 J.Pharm.Chem CODEN: JPCOCM

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)
Indexed in Chemical Abstract
www.stfindia.com

ISSN 0973 – 9874 J.Pharm.Chem CODEN: JPCOCM

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)

Volume 6 • Issue 1 • January – March 2012

Contents

❖

VIEWS

ISSN 0973 – 9874

Spectrophotometric Determination of Aceclofenac Via Complexation with Surfactant

MADHURI D[1], CHANDRASEKHAR KB[1], SOMASEKHAR G[2] AND HARINADHA BABA K[3]

1. Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapur, A.P, INDIA.*
2. Faculty of Pharmacy, Omar-Al-Mukhtar university, Tobruk, Libya.
3. Rahul Institute of Pharmaceutical Sciences, Papaipalem Road, Chirala, A.P. India.

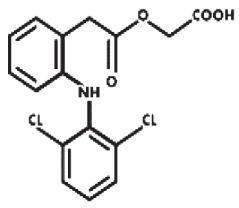
ABSTRACT

A simple, quick and sensitive U-V spectrometric method has been developed for the quantitative estimation of Aceclofenac in bulk and pharmaceutical formulation. The method was based on the complexation with neutral surfactant Tween-20 in aqueous phosphate buffer at pH 7.4 which showed an absorption maximum at 285nm and obeyed Beer's law in the concentration range 2-14µg/ml which was based on the formation of a U.V sensitive complex. The limit of detection and quantification were calculated and relative standard deviations were less than 0.01. The results of analysis for the method have been validated statistically and by recovery studies the results obtained with the proposed method were in agreement with the labeled amounts.

Key words : Aceclofenac, Spectrophotometer, Tween-20,Phosphate buffer.

Introduction

Aceclofenac[1-3] is [[[2-[(2,6,Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid.



It appears as white or almost white crystalline powder. It is practically in soluble in water, freely soluble in acetone, soluble in alcohol. It's Melting point is 148 – 152°C

It is a novel nonsteroidal anti-inflammatory drug indicated for symptomatic treatment for pain and inflammation[7-11].

Numerous double bind, randomized, comparative clinical trials are established. The efficiency and tolerability of aceclofenac compared with diclofenac, ketoprofen, indomethacin, naproxen, piroxicam, and temoxicam in the treatment of ankylosing spondylitis, oestoarthritis, and rheumatoid arthritis. It has shown to be also effective in acute pain conditions involving dental and gynecological pain.

Address for correspondence

Mechanism of Action[4]:

It is known to exhibit multifactor mechanism of actions like.

1. Directly blocks PGE-2 secretion at the site of inflammation by inhibiting 1L-Beta and TNF in inflammatory cells (intercellular action)
2. Stimulates the synthesis of extra cellular matrix of the human articular cartilage.
3. Inhibit neutrophil adhesion and accumulation at inflammatory site in early phase and thus block the pro-inflammatory action of neutrophils.
4. Aceclofenac is a non-steroidal anti-inflammatory drug with greater COX-2 specificity when compared to diclofenac sodium.

The literature survey reveals that Aceclofenac has been estimated by simultaneous estimation[5] densitometry[6], spectrophotometry[7-11], chromatographic[12-15] methods have been reported. No spectrophotometric methods in aqueous media are cited in the literature. We report a simple and sensitive micelle based spectrophotometric sensitive complex using surfactants in aqueous buffer for the analysis of Aceclofenac[16-22].

Experimental

Apparatus

All absorption spectras were made using PERKIN ELMER U.V Spectrophotometer equipped with 10mm matched Quartz cells.

Reagents and Chemicals: All reagents and chemicals used were of Analytical grade. Hydrochloric acid, Potassium dihydrogen phosphate, sodium hydroxide, Tween-20 , Tween -80 and doubly distilled water.

Marketed formulations

Dolokind – Aceclofenac (100mg) – Mankind Ltd.

Experimental procedure

Selection of solvents: The solvent was selected by determining the solubility of Aceclofenac in various solvents such as 0.1 N sodium hydroxide,0.1N Hydrochloric acid pH: 3.2 Acid buffer, pH:4.0 Acid buffer, pH: 5.8 Phosphate buffer, pH: 6.8 Phosphate buffer, pH:7.4 Phosphate buffer, pH 7.4 phosphate buffer +Tween20(0.1%) and pH 7.4 Phosphate buffer +Tween 80(0.1%) The results were given in Table No:1.

Complexation of Aceclofenac with surfactant: An accurately weighed quantity about 100mg Aceclofenac was taken in 100ml volumetric flask and was dissolved in pH 7.4 Phosphate buffer containing 0.1% Tween 20 and volume was made up to the mark. To get the concentration of 1mg/ml, the aliquots portion of stock solution of Aceclofenac was diluted with pH 7.4 Phosphate buffer containing 0,1% Tween-20 to obtain a concentration of 10µg/ml . Measure the absorbance of the complex by scanning between 200nm to 400nm against a blank. The λ max of the complex was obtained at 285nm. Fig No:2

Procedure for dosage form

An accurately weighed amount of freshly powdered tablet equivalent to 100mg of the drug was dissolved in a 20ml of pH 7.4 Phosphate buffer containing 0.1% Tween-20 and after 15minutes of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper and necessary amount of filtrate was added and diluted to 100ml and then the same procedure was followed as above.

Table 1
Effect of solvent on absorption intensity of Aceclofenac in presence of Tween 20 and Tween 80

Solvent	Absorbance in Tween20	Absorbance inTween80
0.1 Hydrochloric acid	0.15	0.05
0.1n Sodium hydroxide	0.05	0.03
Phosphate buffer P^H3.2	0.07	0.01
Phosphate buffer P^H4.0	0.15	0.09
Phosphate buffer P^H5.2	0.18	0.13
Phosphate buffer P^H6.8	0.25	0.25
Phosphate buffer P^H7.4	0.75	0.51
Phosphate buffer P^H9.0	0.64	0.32
Phosphate buffer P_H10.0.	0.64	0.31

Optimization of reaction conditions

Effect of pH and surfactant: At acidic pH there was formation of turbidity and at basic pH the solubility of Aceclofenac was poor, so the solubility was enhanced by using surfactants such as anionic surfactants and neutral surfactants. Aceclofenac showed complete solubility with a neutral surfactant such as Tween-20 and Tween-80 but Tween-20 gave a very clear solution which is highly U.V sensitive than Tween-80.At the pH 7.4 phosphate buffer containing 0.1% tween-20 gave max absorbance value. Fig No:3

Effect of concentration of surfactant: To 1ml of the Aceclofenac stock solution, aliquot portion of 0.01% to 0.05% of Tween -20 in pH 7.4 phosphate buffer is added and make up the volume to 100ml and absorbance values at 284nm were taken. Investigation of the surfactant concentration revealed that 0.1% to 0.5% of Tween-20 was found to be optimum for maximum complexation of Aceclofenac using 100µg/ml concentration. Fig No: 4

Linearity range and quantification procedure:

Beers law was found to be obeyed in the concentration range 2µg to 14 µg/ ml at 295 nm. A(1%,1c.m) equal to $4.55x10^2$ L $mole^{-1}cm^{-1}$.The drug surfactant complex absorbances were fitted to the equation Y=a+bx, where Y is the absorbance at relevant maximum,b is the slope and a is the intercept of the calibration curve. The correlation coefficient was found to be greater than 0.99 indicating exact linearity. The accuracy of the proposed procedure were 100.03 and 100.04. Repeatability and reproducibility were evaluated. Table No:2

Effect of common excipients on analysis of Aceclofenac:

Aceclofenac was mixed with necessary amounts of common excipients and dissolved in a 20ml of pH 7.4 Phosphate buffer containing 0.1% Tween20 and after 15minutes of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter

Table 2
Statistical analysis of calibration graph and analytical data for complexation of Aceclofenac with Tween 20 in Phosphate buffer pH7.4

Parameter	Data
wavelength2ûmax	285
Beer'slaw limit µg/ml	14-Feb
Molar absorptivity (•) $Lmol^{-1}cm^{-1}$	$4.55x10^2$
Regression equation	
a)slope(b)	0.075
bIntercept(a)	0.037
correlation coefficient(r2)	0.999
Detection limit µg/ml	0.9
Quantification limit µg/ml	2.73
RSD%	0.01

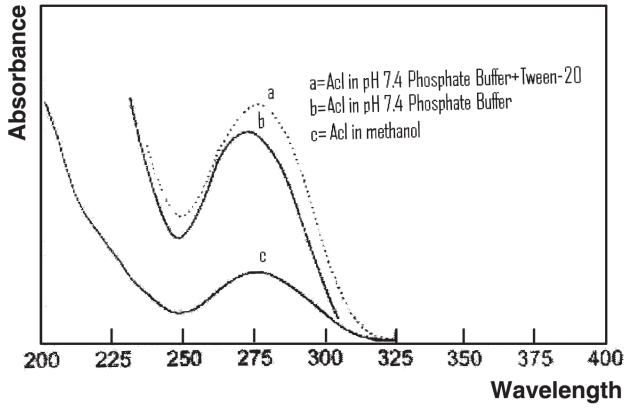


Fig.1 : Absorption spectras of Acl in different solvents.

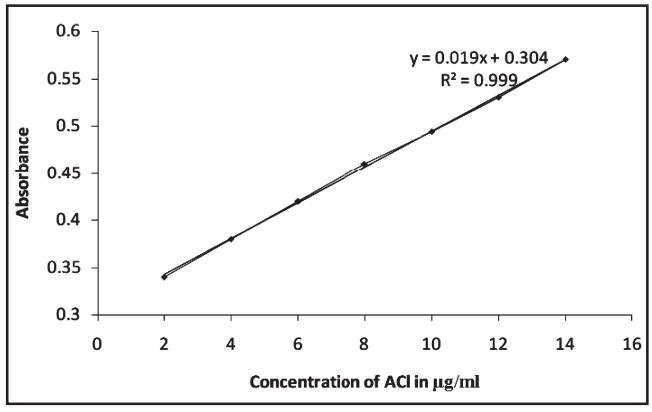
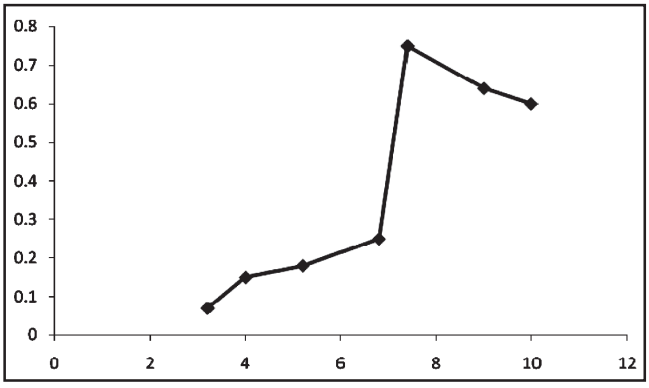


Fig.2 : Beers lamberts plot of Acl.

paper and necessary amount of filtrate was added and diluted to 100ml and then the same procedure followed as above. Table No:3

Assay of dosage form:

An accurately weighed amount of freshly powdered tablets equivalent to 100mg of the drug was dissolved in a 20ml of pH 7.4 Phosphate buffer containing 0.1% Tween20 and after 15minutes of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper and necessary amount of filtrate was added and diluted to 100ml and then the same procedure followed as above. Table No:4



x-axis- pH of Phosphate buffer; y-axis- Absorbance

Fig.3 : Effect of pH on absorption intensity of Aceclofenac-Tween20 complex.

Table 3

Analysis of Aceclofenac in presence of common excipients using the proposed method

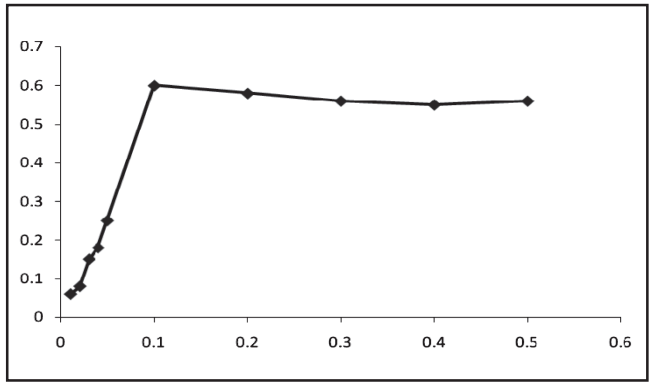
Ingredient	Recovery ±S.D%
Glucose(50mg)	100.01±0.01
Lactose(10mg)	100.03±0.0163
Magnesium sterate(10mg)	99.8±0.138
Starch(10mg)	100.01±0.0075

Results and Discussions:

The Linearity range of Aceclofenac-Tween -20 complex at pH 7.4 covered over a range of 2-14µg/ml of the drug with A(1%1cm)equals to $4.55x10^{2}Lmol^{1}cm^{-1}$.The drug surfactant complex absorbances were fitted to the equation Y=a+bx, where Y is the absorbance at relevant maximum ,b is the slope and a is the intercept of the calibration curve. The correlation coefficient was found to be 0.999 indicating exact linearity. The accuracy of the proposed procedure were 100.03 and 100.04. Repeatability and reproducibility were evaluated.

Conclusion:

The proposed procedure is stability indicating aqueous method which can be used for the determination without



x-axis Concentration of surfactant, ; y-axis Absorbance

Fig.4 : Effect of Concentration of surfactant on the absorbance intensity of Aceclofenac–Tween20 complex

Table 4
Determination of Aceclofenac in pharmaceutical dosage forms

Sample	Recovery±R.S.D% Proposed method
Dolokind	100.03±0.008163
Dolokind	100.04±0.01

interference in dosage form. The drug being poorly water soluble, the solubility has been enhanced by complexation with surfactant and considered more selective in determining the intact drug. In addition, there is background interference of the complex matrix and the method resolved the individual drug, drug additives and drug decomposition both interfered.

References:

1. The Drug Profile, CIMS, Current Index of Medical Specialities, India,Jul-2004, 15
2. The United States Pharmacopoeia 24/ National Formulary 19, The United States Pharmacopoeial Convection, Rockville, 2000, 2116.
3. Sweetman, S.C., In; Martindale The Complete Drug Reference, 33rd Edn., Pharm. Press, London, 232
4. Kasture, V.S., Bagat, A.D., Puro, N.C. and More, R. S., Spectrophotometric method for simultaneous estimation of Ofloxacin and Ornidazole in Tablet dosage form, Indian Drugs, 2004, 41, 51-53
5. Wilson and Gisvold's, In; Text Book of Organic Medicinal and Pharmaceutical Chemistry, 10th Edn., Lippincott Williams and Wilkin, 10 edn 1998.
6. Y. S. El-Saharty, M. Refaat, S. Z. El-Khateeb, "Stability-indicating spectrophotometric and densitometric methods for determination of aceclofenac", Drug Dev. and Ind. Pharm., 28(5), 2002, 571-582.
7. Aceclofenac and Paracetamol from solid dosage form employing Simultaneous equation and Derivative Spectrophotometry", Ind. J. Pharm. Sci., 1997, 45-48.
8. Srinivasan K. K., Shirwaikar A., "Simultaneous estimation of Aceclofenac and Paracetamol in solid dosage form by Ultraviolet Spectrophotometry". Indian Drugs 43(2) 2006, 141-145.
9. Shanmugam S., Cendil Kumar A., "Spectrophotometric method for the estimation of aceclofenac in tablets", Indian Drugs 42(2) 2005, 106-107.
10. S.Appala Raju, Manzoor Ahmed, Y. N. Manohara, "Spectrophotometric determination of aceclofenac in pharmaceutical dosage form", The Indian Pharmacist, Nov. 2005, 102-103.
11. Y. S. El-Saharty, M. Refaat, S. Z. El-Khateeb, "Stability-indicating spectrophotometric and densitometric methods for determination of aceclofenac", Drug Dev. and Ind. Pharm., 28(5), 2002, 571-582.
12. Hasan N. Y., Elzeany B. E., Wagieh N. E., "Stability indicating methods for the determination of aceclofenac", IL Farmaco, 58, 2003, 91-99.
13. Lahiri K., Kishor Babu G., "Simultaneous determination of Diclofenac sodium and Paracetamol in pharmaceutical preparation by HPLC", The Eastern Pharmacist, vol. xxxv No. 414, June 1992, 117-119.
14. Satish Joshi, Yateen Shah, K. C. Jindal, "Simultaneous determination of Diclofenac sodium and Paracetamol in tablets by HPLC", The Eastern Pharmacist, vol. xxxv,410, 1992, 113-114.
15. The estimation of Aceclofeac in tablet dosage form", Indian Drugs,42(10) October 2005, 693-695.
16. Changchuan guo, Peng Dong, rapid spectrophotometric determination of gatifloxacin using europiumsensitized method Luminescence 3,(1), 7-13.
17. Abdellate H.E ., J.Pharm ,biomed,Anal., 17, 1998, 167-171.
18. Abdineh.H.,ELYazbic F.A.,Blaih S.M.,Shalaan R.A., spectrosc.Lett., 31.1998,99-980
19. EL.Shabouri.S.R.,Emara K.M., Khashaba P.Y., Mohammed A.M ., Anal. Lett., 31, 1998, 1367-1387.
20. Liptay W., Briegleb G., Schnindler K., Z. Elelcktrochem., 66, 1962,331-341.
21. Martin A.N.,\Swarbrick J.,Cammrata A." Physical Pharmacy" 3ed., Lee&Febiger, Philadelphia, P.A, 1969. 344.
22. International conference on Harmonization (ICH), Draft guidelines on validation of analytical procedure- definition and terminology, Federal Register, 1995,11260.

ISSN 0973 – 9874

Isolation and characterization of β-Steroids and Quercetin form Talinum *cuneifolium*

GAJULA SANTHOSH KUMAR RAJA[1*], NARENDRA REDDY G[2], JAYAVEERA KN[3] AND YOGANANDA REDDY K[4]

1. Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapur-515002.
2. Lecturer in Chemistry, Govt. Polytechnic for Women, Suryapet - 508213, Nalgonda(Dist) A.P.
3. Professor& Head, Jawaharlal Nehru Technological University Anantapur, Anantapur-515002.
4. International Science-Tech Research Institute, Anantapur.

ABSTRACT

Present work is focused on phytochemical, isolation and characterization studies, of medicinal plant *Talinum cuneifolium* belonging to the family *portulacaceae.* The dry powder of plant parts was extracted with various solvents by using soxhlet apparatus. These phytochemical screening showed the presence of flavonoids, glycosides, saponins and steroids and absence of alkaloids, phenols, lignin, tannins, terpenoids, quinones, fixed oils and volatile oils. Isolation of secondary metabolites was done by using column chromatography using column grade silica gel, and different fractions were collected using gradient elution technique. The isolation and purification afforded white crystalline powder and yellow powder was subjected to physical, chemical and spectral the selected fraction were characterized by using IR, 1H NMR and MASS spectroscopy. The secondary metabolites were concluded as β- Steroids and Quercetin.

Introduction

Plants play an important role in the biosynthesis of plant chemical, these divided into two major categories: primary metabolites and secondary metabolites. Primary metabolites are substances produced by all plant cells that are directly involved in growth, development, or reproduction. Examples include sugars, proteins, amino acids, and nucleic acids. Secondary metabolites are not directly involved in growth or reproduction but they are often involved with plant defense. These compounds usually belong to one of three large chemical classes: terpenoids, phenolics, and alkaloids. Secondary metabolites do not seem to be vital to the immediate survival of the organism that produces them. Secondary metabolites are of important class of plant chemicals which are used as traditional medicine for cure of much disease, since for the existence of mankind. With the development of natural product chemistry, the potential of chemotaxonomy is now becoming increasing by obvious.

Talinum cuneifolium belong to the family portulacaceae, distribution: As a weed in wate places, Found in India, Nepal, and Africa Synonyms: Orygia portulacifolia Forssk. Portulaca cuneifolia, Vahl. *Talinum portulacifolium*, Asch. ex Schweinf. Description: Perennial succulent herb, upto1mt tall stem sprawling from the thickened root, or sometimes semi-scandent . Leaves slightly fleshy, broadly obovate to obovate-oblong, 2–8 cm x1–3.5 cm , cuneate sub sessile, apex broadly rounded, apiculate. Inflorescence a terminal panicle, or appearing racemose, the flowers in lateral cymes on a central axis 7–30 cm long, individual cymes 1–few flowered, subsessile or stiffly pedunculate; pedicels 1–2 cm long, recurving in fruit. Flowers 2–2.5 cm across, sepals broadly ovate, concave, thinly membranous, 4–5.5 mm long, apiculate; petals obovate, usually magenta, sometimes crimson, stamens more than 20, filaments magenta, anthers yellow; ovary green. Capsule globose, dull yellowish, 6–7 mm high; seeds subreniform. The leaves are used as leafy vegetable.

Phytosterols are plant steroids with a chemical structure similar to that of cholesterol ($C_{27}H_{46}O$), a steroid found in animals. Cholesterol is essential to human biology and is naturally produced in the human body, but excess levels of dietary cholesterol can damage the circulatory system. Phytosterols have a demonstrated ability to reduce cholesterol levels in the human bloodstream because their similar chemical structure allows them to react with chemicals in the digestive tract that normally bond with

*Address for correspondence
Email: santhoshchem08@gmail.com

cholesterol so that the cholesterol can be absorbed by the intestines. When phytosterols are present, they bond with these chemicals and prevent cholesterol molecules from doing so, causing dietary cholesterol in the intestines to be excreted rather than absorbed. Consequently, phytosterol-rich foods can have a beneficial effect on people with elevated cholesterol levels. The most naturally phytosterol-laden foods are vegetable oils, nuts, and things made from them. Other foods can be artificially enriched with phytosterols, and they can also be taken in the form of tablets or pills. There is also research suggesting that phytosterols consumption can decrease the risk of some cancers, such as lung, breast, and stomach cancer. Excessive phytosterol levels may also interfere with the absorption of some dietary nutrients and can have dangerous effects in people with certain health conditions, such as the metabolic disorder sitosterolemia, so intentionally increasing phytosterol intake for health reasons without first seeking guidance from a physician is not recommended.

Quercetin is the most abundant of the flavonoids. Quercetin belongs to the flavonoids family and consists of 3 rings and 5 hydroxyl groups. Querctin is also a building block for other flavonoids. Quercetin occurs in food as a aglycone (attached to a sugar molecule). Only a small percentage of the ingested quercetin will get absorbed in the blood. Quercetin, a member of the flavonoids family, exerts many beneficial health effects, including improvement of cardiovascular health, reducing risk for cancer, protection against osteoporosis. This phytochemical has anti-inflammatory, anti-allergic and antitoxic effects. Most of these properties are linked to its strong antioxidant action of quercetin but quercetin also modulates the expression of specific enzymes. Quercetin induces apoptosis and influences protein and lipid kinase signaling pathways. Quercetin is a candidate for preventing obesity-related diseases.

Experimental

Collection, Identification and preparation of plant materials

The plant were collected from local area of Anantapur, The plant was taxonomically identified, authenticate by HOD, Botany Department, SK university Anantapur. The parts of the plant were manually separated was air dried, powdered, sieved, weighed and stored in air tight container and subsequently referred to as powdered drug.

Extraction and Isolation

Powdered (400g) plant parts of *Talinum cuneifolium* was defatted exhaustively with petroleum ether (60 & 80°c) and methanol in a soxhlet extractor. The solvent was recovered under pressure to obtained dark greenish brown oily mass (5.6g), which was labeled as petroleum ether extracts (PEE) and kept in the refrigerator. The resulting marc was air dried at room temperature and then exhaustively extracted successively with solvents with increased polarity and concentrated under reduced pressure and labeled accordingly. The petroleum ether extract of aerial parts of the plant was saponified using 1M alcoholic KOH, to remove fatty material and then subsequently picked up in petroleum ether and the solvent was evaporated to yield 3g of unsaponified matter. This fraction contains lesser number of components than the unsaponified extract. Methanolic extract was used for the isolation of flavonides.

Chromatographic separation

Isolation and characterization of β- Steroids:

A small quantity of unsaponifiable matter was dissolved in chloroform and this solution is spotted on TLC plates using precoated aluminium with silica gel 60 F254. Then the TLC plates were run by specific solvent system and viewed individually under UV light and also (5%) sulphuric acid in methanol reagent. Through several pilot experiments it was found that the compounds of unsaponifiable fraction were separated by the solvent system of ethyl acetate and chloroform and ethanol in the proportion of (0.5: 6.4: 3.1 v/v/v). The chromatograms when developed in iodine chamber yielded six to seven spots respectively and three spots at R_f (0.34, 0.56, 0.82) becomes reddish brown soon turns to purple or violet indicate zones for steroidal nucleus. Column chromatography of PEE was conducted using silica gel (Mesh 60 120) that was packed using wet packing method in hexane. The column was run using a mixture of hexane, ethyl acetate by gradient elution technique. TLC was used to monitor the eluates. A total of 158 eluates were collected. Similar fractions were pooled together. Further purification is carried out using preparative TLC. Spots were identified, scraped and eluates using petroleum ether and chloroform as solvents [1, 2].

Finally eluate ST yielded a single spot when subjected to TLC using several solvent systems including ethyl acetate and chloroform and ethanol in the proportion of (0.5: 6.4: 3.1 v/v/v), ethyl acetate: ethanol (7.3:2.7), chloroform: ethyl acetate (9:1) and it showed to be homogenous compound. ST a white crystalline powder **compound 1** (100mg) with melting point (137-140°C) was further subjected to IR, Proton NMR (400MHz), Carbon 13 NMR (100 MHz) and MASS to ascertain the chemical structure.

Isolation and characterization of Quercetin.

The methanolic extract of *Talinum cuneifolium* was taken and subjected for the column chromatographic method, the column is packed with the silica gel slurry (60-120 mesh size) prepared in petroleum ether, 3 gm of methanolic extract was loaded on the column and the column was then eluted with various solvent like petroleum ether, benzene, chloroform, and acetone, ethanol, methanol and water in order of their increasing the polarity in the ration of (1:1) v/v. Actone fraction results in a clear precipitation of white color crystalline compound, the methanolic fraction kept in the refrigeration results in the

precipitation of yellow powder which further purified, the isolated compound are spoted in a pre-coated TLC pates for ascertains of spots using ethylacetate : formic acid : glacial acetic acid: water: chloromethane (9:1.5:0.5:3 v/v/ v/v) and the melting point of the compound was determined the isolated compound was subjected for IR, Proton NMR (400MHz), Carbon 13 NMR (100 MHz) and MASS to ascertain the chemical structure.

Results and Discussion

The extracted of **Talinum *cuneifolium,*** were subjected for various phytochemical tests and the petroleum ether and methanolic extracts showed the presence of flavonoids, glycosides, saponins and steroids and absence of alkaloids, phenols, lignin, tannins, terpenoids, quinones, fixed oils and volatile oils. cssThe selected extracts were subjected for column separation and the isolated compound was subjected for IR, Proton NMR (400MHz), Carbon 13 NMR (100 MHz) and MASS to ascertain the chemical structure.

IR spectra of isolated compound 1. IR (KBR), OH: 3426, 1056 cm^{-1}, Hydrogen Skeleton: 2935, 2852, 1706, 1462 cm^{-1}, -CH=C=:965, 802 cm^{-1} C=C stretching however this band is weak, at 1457.3 cm 1 is a bending frequency for cyclic (CH2)n and 1381.6 cm 1 for –CH2(CH3)2 γ. The absorption frequency at 1038 cm 1 signifies cycloalkane. The out of plane C H vibration of unsaturated part was observed at 881cm 1.

NMR spectra of isolated compound 1 : ^{1}H NMR (DMSO-d_6).δ 0.8-2.3: (46 H), δ 3.5: (m, 2H, =CH- CH_2-), δ 5.6: (d, 1H, -CH=C=)

^{13}C NMR: 13C NMR has shown recognizable signals 145.2 and 121.7 ppm, which are assigned C5 and C6 double bonds respectively as in Δ5 Beta steroids 11. The value at 19.32 ppm corresponds to angular carbon atom (C19). Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at δ145.2, 139.8, 121.7 and 118.89

Mass spectrum of isolated compound 1: Mass spectrum (EI. MS): m/z 414 (M+), m/z 396 (M-18), 383 (M-33), 273 (M-side chain), 255 (M-side chain-18), 231 (M-side chain-42), 213 (231-H_2O). All the recorded data such as IR, ^{1}HNMR, 13 C NMR and mass spectrum conclusively prove that the isolated steroidal compound may be β-SITOSTEROL, which was confirmed by standard reference compound and compound data base. [3-5]

IR spectra of isolated compound 2:

IR (KBR)-OH: 3450 cm^{-1}, -C=O: 1645 cm^{-1}, -C=C=: 1610, 1505 cm^{-1}

NMR spectra of isolated compound 2:

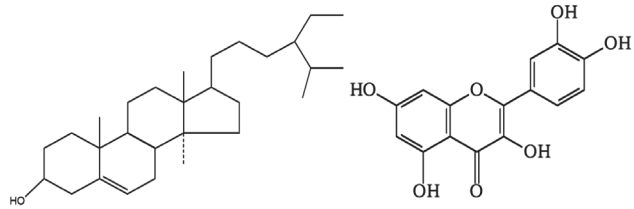
^{1}H NMR (DMSO-d_6). δ 3-3.4: (BS, 5H, -OH), δ 6.2: (d, 1H, -H-6), δ 6.4: (d, 1H, -H-8), δ 6.8 : (d, 1H, -H-5'), δ 7.5: (d, 1H, -H-6'),δ 7.6 : (d, 1H, -H-2')

Mass spectrum of isolated compound-2:

Mass spectrum (EI. MS): m/z 303.11 (M+) All the recorded data such as IR, ^{1}HNMR and mass spectrum conclusively prove that the isolated compound it may be Quercetin, farther it is confirmed by using standards Quercetin.[6-7].

Conclusion

From the above findings, β sitosterol and Quercetin were isolated from petroleum ether extract and methanolic extracts of the plant parts of *Talinum cuneifolium* and chemical structures elucidated respectively. It was carried out by means of various physical (solvent extraction, TLC, Column chromatography) and spectral techniques.



β-sitosterol Quercetin

Reference

[1]. Habib MR., Nikkon F., Rahman ME., and Karim MR. Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantean* (Linn). *Pak. J. Biol. Sci*. 2007; 10: 4174 4176.

[2]. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., chapman and Hall: London. ISBN: 0 412 57270 2, 1998: 302.

[3]. Okunade AL. Ph.D Thesis, University of Ibadan. Nigeria. 1981; 84.

[4]. Abbiw DK. Useful plants of Ghana, Intermediate Tech. Publication. London: Royal Botanic Gardens, Kew. 1990: 207.

[5]. Agarwal PK., Jain DC., Gupta RK., and Thakur RS. Carbon 13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry. 1985; 24: 2476 2496.

[6]. Shoskes DA, Zeitlin, Shahed A, Rajfer J. Quercetine in men with category III chronic prostatitis. A preliminary prospective double-blind, placebo-controlled trial.. Urology, 1999: 54:960-63.

[7]. Cruz-correa M, Shoskes DA, Sanchez, Comination treatment with curcumin and Quercetin of adenomas in familial adenomatous polyposis. Clinical Gastroenterology & Hepatology. 2006: 4: 1'035-38.

[8]. Flora of Presidency of Madras by J.S.Gamble. e floras.ENVIS;This species is not recorded in Flora of Nellore district by B.Suryanarayana, and A.S.Rao.

ISSN 0973 – 9874

Synthesis And Biological Activity of Some Novel Pyrazolyl and Isoxazolyl Aryl Azo 1, 8-napthyridines

SUBRAMANYAM S[1], RAJA S[2] AND JAYAVEERA KN[3]

1. Bharat Institute of Pharmacy, Mangalpally(Village), Ibrahimpatnam, Ranga Reddy (Dist), Andhra Pradesh, India
2. Gitam Institute of Pharmacy, Gitam University, Gandhi nagar, Rushikonda, Visakhapatnam, Andhra Pradesh, India
3. Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh,India

ABSTRACT

Napthyridinylaryl azopyrazoles and azoisoxazoles have been synthesized in two stages. In the first stage, hydrazones (III&IV) were synthesised by coupling a diazonium salt of P-(aminophenyl)-1.8-napthyridine with active methylene compounds like acetyl acetone and dibenzoyl methane. In the second stage hydrazones (III&IV) upon treatment with hydrazine and substituted hydrazines in acetic acid to gave the corresponding substituted pyrazoles (IIa-c & IIIa-c) and with hydroxylamine HCl afforded corresponding substituted azoisoxazoles (V & VI). The synthesized compounds derivatives have been characterized on the basis of IR, H ^{1}NMR and mass spectral data and these were screened for anti-inflammatory activity and analgesic activity**.**

Keywords: 1, 8-Napthyridines, anti-inflammatory and analgesic activity.

Introduction

1, 8-Napthyridine derivatives posses various types of biological activities including antibacterial [1,2] diuretic [3], antimalarial [4], anti-inflammatory [5], and antihypertensive activity. Larger numbers of pyrazole derivatives have been synthesized as potential antibiotics, antidiabetics, antineoplastics and bacteriostatics [6]. Compounds like azo or hydrazono group have been found to exhibit a wide range of biological activities [7, 8]. Isoxazole have been repeatedly shown as useful synthons in organic synthesis and posses a broad spectrum of biological activities such as aniviral[9], antitubercular,[10] analgesic, antiprotozoal etc. Isoxazole also serve as an important building block for the synthesis of biological active molecule and serve as prodrug for an arthritic agent[11].

Materials and Methods

Melting points were determined in open capillaries in electrical apparatus and are Uncorrected. IR spectra were recorded on a FT-IR spectrometer using KBR pellet. The NMR spectra were recorded in JMR spectrometer using TMS as internal standard. The Mass spectra were recorded in NCMS spectrometer. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass. The spots were developed in iodine chamber and visualized under ultraviolet lamp.

Address for correspondence

Experimetal

I. ***General procedure for preparation of intermeadiate hydrazono derivatives (II&III)***

Scheme-1.

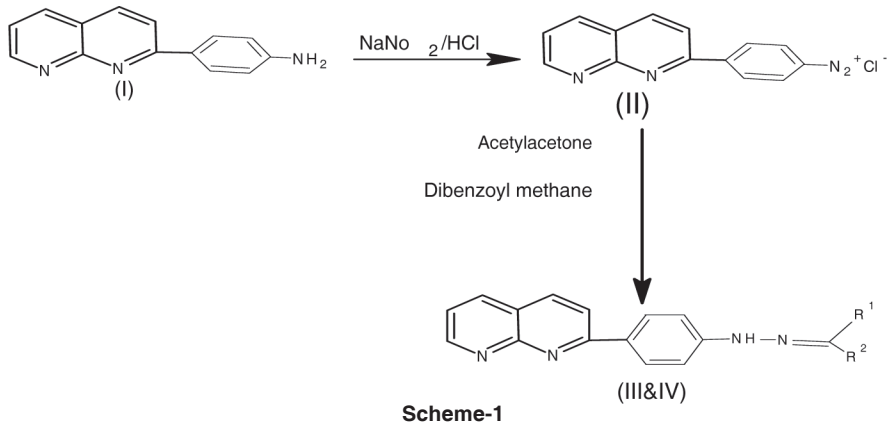
Preparation of intermediate hydrazono compounds (III &IV)

1. Preparation of 3-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono}pentane-2,4-dione (III)

2-(p-aminophenyl)-1,8-napthyridine(1) (0.01mol) was dissolved in a mixture of HCl (8ml) and water (6ml) then cooled to 0Ú in an ice bath and a cold aq solution of sodium nitrite (0.03) was added. The diazonium salt solution was filtered directly into a cold solution of acetyl acetone (0.01mol) and sodium acetate (0.122mol) in ethanol. The resulting yellow solid (II) was washed with water and then recrystallized from ethanol

2. Preparation of 2-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono}-1,3-diphenyl-propane-1,3-dione(IV)

2-(p-aminophenyl)-1,8-napthyridine(1)(0.01mol) was dissolved in a mixture of HCl (8ml) and water (6ml) then cooled to 0Ú in an ice bath and a cold aq solution of sodium nitrite (0.03) was added. The diazonium salt solution was filtered directly into a cold solution of dibenzoylmethane (0.01mol) and sodium acetate (0.122mol) in ethanol. The



Scheme-1

III-R^1= $COCH_3$ R^2= $COCH_3$ IV - R^1= COC_6H_5 R^2= COC_6H_5

resulting yellow solid (II) was washed with water and then recrystallized from ethanol.

Scheme-2 :

Procedure for preparation of title compounds (IIIa-d &V)

3. **Preparation of 3,5-dimethyl 4-(napthyridin-2-yl phenyl azo) pyrazoles (IIIa)**

3-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono} pentane-2, 4-dione (III) (0.002mol) was dissolved in glacial acetic acid (20ml) and a solution of hydrazine in glacial acetic acid (10ml) was added and the mixture was refluxed for 4hr and then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol.

4. **Preparation of 1-phenyl - 3,5-dimethyl-4-(napthyridin-2-yl phenyl azo)-pyrazoles(IIIb)**

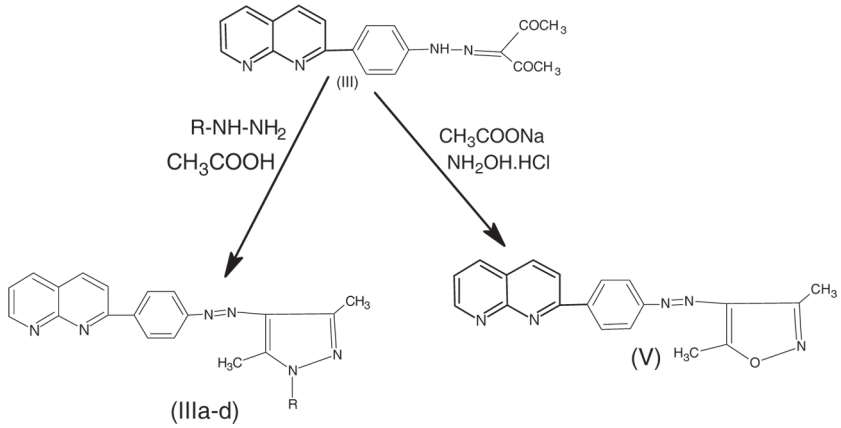
3-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono} pentane-2, 4-dione (III) (0.002mol)was dissolved in glacial acetic acid (20ml) a solution of phenyl hydrazine in glacial acetic acid was added and the mixture was refluxed for 4hr,and then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol.

5. **Preparation of 1-Thiocarbamoyl - 3,5-dimethyl-4-(napthyridin-2-yl phenyl azo)-pyrazoles (IIIc)**

3-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} pentane-2,4-dione (III) (0.002mol)was dissolved in gl acetic acid(20ml) a solution of Thiosemicarbazide in gl acetic acid was added and the mixture was refluxed for 4hr and then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol

6. Preparation of 1-Isonicotinyl- 3,5-dimethyl -4-(napthyridin-2-yl phenyl azo)-pyrazoles (IIId)

3-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} pentane-2, 4-dione (III) (0.002mol) was dissolved in glacial acetic acid (20ml) and a solution of Isoniazide in glacial



Scheme-2

IIIa —R= H, IIIb- R= C_6H_5, IIIc - R= $CSNH_2$, IIId- R= COC_5H_4

acetic acid was added and the mixture was refluxed for 4hr,and then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol

7. Preparation of 3,5-dimethyl-4-(napthyridin-2-yl phenyl azo)-Isoxazoles (V)

2-**{[**4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-1,3-diphenyl-propane-1,3-dione (IV) was dissolved in ethanol. A solution of sodium acetate (1g) and hydroxylamine HCl and water was added to it. It was then refluxed for 4hrs. The resulting solid (V) was obtained which was recrystallized from ethanol.

Scheme-3 General procedure of preparation of Title compounds (IVa-d &VI)

8. Preparation of 3,5-diphenyl napthyridinyl azo pyrazoles (IVa)

2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-1,3-diphenyl-propane-1,3-dione(IV) was dissolved in gl acetic acid(20ml) a solution of hydrazine in glacial acetic acid was added and the mixture was refluxed for 4hr, then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol.

9. Preparation of 1-phenyl-3,5-dimethyl 4-(napthyridin-2-yl phenyl azo) pyrazoles (IVb)

2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-1,3-diphenyl-propane-1,3-dione(IV) was dissolved in glacial acetic acid(20ml) a solution of phenylhydrazine in glacial acetic acid was added and the mixture was refluxed for 4hr.then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol

10. Preparation of 1-thiocarbamoyl - 3,5-diphenyl- 4-(napthyridin-2-yl phenyl azo)-pyrazoles (IVc)

2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-1,3-diphenyl-propane-1,3-dione(IV) was dissolved in glacial acetic acid (20ml) a solution of thiosemicarbazaide in glacial acetic acid was added and the mixture was refluxed for 4hr, then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol

11. preparation of 1-Isonicotinyl-3,5-diphenyl-4-(napthyridin-2-yl phenyl azo)-pyrazoles (IVd)

3-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono} pentane-2,4-dione (III) (0.002mol)was dissolved in glacial acetic acid(20ml) a solution of Isoniazide in glacial acetic acid was added and the mixture was refluxed for 4hr and then it was cooled and allowed to stand overnight . The separated solid was filter, dried and then recrystallized from ethanol

12. Preparation of 3,5-diphenyl-4-(napthyridin-2-yl phenyl azo)- Isoxazoles (VI)

2-**{[**4-(1, 8-naphthyridin-2-yl)phenyl]hydrazono}-1,3-diphenyl- propane-1,3-dione (IV) was dissolved in ethanol. A solution of sodium acetate (1g), hydroxylamine HCl and water was added to it. It was then refluxed for 4hrs. The resulting solid (V) was obtained which was recrystallized from ethanol

Acute toxicity Studies

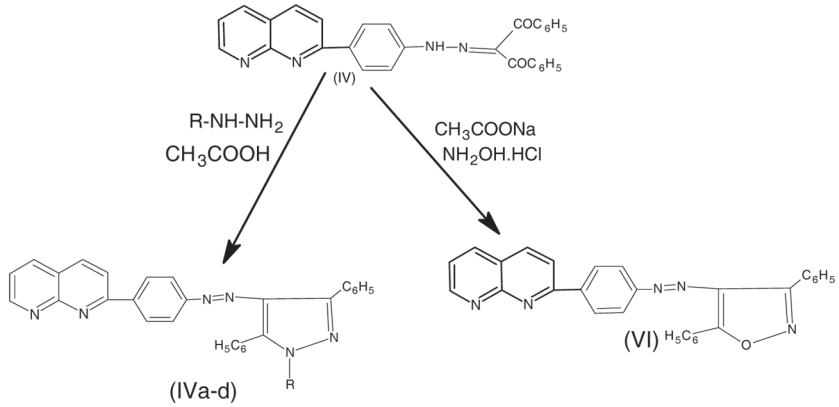
The acute toxic study was done according to the OECD guidelines on Acute Oral Toxicity under a computer guided statistical programme-AOT423 stat programme; the animals were monitored for the behavioural changes, weight variation, toxicity and death rate.

Anti-inflammatory Activity

CARRAGEENAN-INDUCED RAT PAW OEDEMA

Experimental Procedure

Oedema was induced by sub planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into the right hind paws of the rats of four groups of six animals



Scheme-3

IIIa —R= H, IIIb- R= C_6H_5, IIIc - R= $CSNH_2$, IIId- R= COC_5H_4N

each. The volume of the injected and contra-lateral paws were measured 1,2,3 and 4 h after induction of inflammation using a plethysmometer according to the method described by Winter et al. (1962) The test groups received the synthesized compounds (200mg/kg), the standard group received phenylbutazone (100 mg:kg), and the control animals received the vehicle only alone (3% V/V tween-80 10 ml/kg) p.o. All the treatments were given intraperitoneally 30 min prior to the injection of carrageenen an except for the synthesized compounds. Increase of paw oedema thickness was calculated (12). The results are expressed as mean ± S.E.M. Dennett's t-test was used to verify the statistical significance at $p<0.05$ between the treated and control groups.

For comparison purpose, the volume of oedema at various prefixed time intervals was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated.

Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{Vo - V_t}{Vo} \times 100$$

Where, Vo = Volume of the paw of control at time't'.

V_t = Volume of the paw of drug treated at time't'.

From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated.

Analgesic Activity

The writhing test described by Koster et al. (1959) was adopted. A total of 42 mice divided into seven groups (n=6) were used and treated as follows; group 1 served as control and received vehicle alone (3% V/V tween-80 10 ml/kg) p.o., groups 2 to 6 received 200 mg/kg p.o. of the synthesized compounds like IIa, IIb, IIc, IId and V respectively, while group 7 received 100 mg/kg of acetyl salicylic acid (standard drug) p.o. Ten ml/kg of 0.7% aqueous solution of acetic acid were given to all mice i.p. 30 min later. Each mouse was placed in a transparent observation cage and abdominal constriction resulting from injection of acetic acid for the period of 20 minutes was counted. Results were presented as percent inhibition of analgesia, calculated as the reduction in the number of writhes between control animals and those pre-treated with either the synthesized compounds or acetyl salicyclic acid.

The values were expressed as mean ± SEM from 6 animals. The results are expressed as mean ± S.E.M. Dennett's t-test was used to verify the statistical significance at $p<0.05$ between the treated and control groups.(14,15)

Results and Discussion

1. Spectral data of 3,5-dimethyl 4-(napthyridin-2-yl phenyl azo) pyrazoles (IIIa)

IR (cm) 3010 (CH_3), 1143 (C-N), 1620 (C=C), 1690 (C=N), 1510-1540 (N=N)

NMR 9.82 (s,1H. NH), 7.52 (m,1H,C_3-H), 7.72 (m,1H,C_4-H), 7.92 (m,1H,C_5-H), 7.48 (m,1H,C_6-H),8.21 (m,1H,C_7-H), 6.9 2.5 (s,3H. 3-CH_3), 2.1(s,3H. 5-CH_3)

2. Spectral data of 1-phenyl- 3,5-dimethyl 4-(napthyridin-2-yl phenyl azo)pyrazoles (IIIb)

IR (cm) 3014 (CH_3), 1150 (C-N), 1626 (C=C), 1690 (C=N), 1540-1560 (N=N)

NMR 7.42 (m,1H,C_3-H), 7.52 (m,1H,C_4-H), 7.81 (m,1H,C_5-H), 7.30 (m,1H,C_6-H),8.42 (m,1H,C_7-H), 6.8 -7.3 (m,8H,Ar-H) 2.67 (s,3H. 3-CH_3), 2.52(s,3H. 5-CH_3)

3. Spectral data of 1-Thiocarbamoyl - 3,5-dimethyl 4-(napthyridin-2-yl phenyl azo)-pyrazoles (IIIC)

IR (cm^{-1}) 3232,3962 (NH_2), 1620 (C=C), 1629 (C=N), 1510-1560 (N=N)

NMR: 7.52 (m,1H,C_3-H), 7.82 (m,1H,C_4-H), 8.12 (m,1H,C_5-H), 7.22 (m,1H,C_6-H),8.56 (m,1H,C_7-H), 6.8 -7.1(m,4H,Ar-H), 2.5 (s,3H. 3-CH_3), 2.1(s,3H. 5-CH_3)

4. Spectral data of 1-Isonicotinyl- 3,5-dimethyl 4-(napthyridin-2-yl phenyl azo)-pyrazoles

IR (cm^{-1}) 3018 (CH_3), 1143 (C-N), 1620 (C=C), 1690 (C=N), 1510-1540 (N=N)

NMR: 9.82 (s,1H. NH), 7.52 (m,1H,C_3-H), 7.72 (m,1H,C_4-H), 7.92 (m,1H,C_5-H), 7.48 (m,1H,C_6-H),8.21 (m,1H,C_7-H), 6.9-7.3 (m,8H,Ar-H) 2.5 (s,3H. 3-CH_3), 2.1(s,3H. 5-CH_3)

5. Spectral data of 3,5-dimethyl- 4-(napthyridin-2-yl phenyl azo)-Isoxazoles (IIe)

IR (cm^{-1}) 3010 (CH_3), 1143 (C-N), 1620 (C=C), 1690 (C=N), 1510-1540 (N=N)

NMR: 9.82 (s,1H. NH), 7.52 (m,1H,C_3-H), 7.72 (m,1H,C_4-H), 7.92 (m,1H,C_5-H), 7.48 (m,1H,C_6-H),8.21 (m,1H,C_7-H), 6.9 2.5 (s,3H. 3-CH_3), 2.1(s,3H. 5-CH_3)

Pharmacological activity

Anti-inflammatory activity

The results of the anti-inflammatory effects of 100mg/kg of compounds IIa,IIb, IIIc,IIId & V on carrageen induce Oedema in rates hind paws are presented in table-1 & figure2. The % inhibitions were 21.86%,38.48%, 36.44,16.90%,25.36 % respectively for compound IIIa, compound IIIb,compound IIIc, compound IIId and compound V respectively with significance value less than 0.05. Compounds IIb & IIc shows moderate activity compared standard drug phenyl butazone (49.85%) where as compound IIId shows weak activity against standard with % inhibition of 16.90%

Table 1

Anti Inflammatory Activity

Compound	1hr		2hr		3hr		4hr	
	Mean ± SEM	%red	Mean ± SEM	%red	Mean ± SEM	%red	Mean ± SEM	%red
control	3.65 ± 0.18	NA	3.54 ± 0.17	NA	3.53 ± O.12	NA	3.43 ± 0.28	NA
standard	2.17 ± 0.18	40.54	2.03 ± 0.19	43.13	1.94 ± 0.17	45.04	1.72 ± 0.15	49.85
II_a	3.32 ± 0.04	9.04	3.14 ± 0.07	12.04	2.81 ± 0.08	20.03	2.68 ± 0.11	21.86
II_b	2.91 ± 0.07	20.27	2.65 ± 0.02	25.77	2.23 ± 0.02	36.82	2.11 ± 0.16	38.48
II_c	2.54 ± 0.16	30.41	2.48 ± 0.14	30.53	2.31 ± 0.11	34.56	2.18 ± 0.10	36.44
II_d	3.45 ± 0.11	5.47	3.31 ± 0.01	7.28	2.90 ± 0.25	17.84	2.85 ± 0.14	16.9
II_e	3.14 ± 0.12	13.97	3.05 ± 0.12	14.56	2.84 ± 0.13	19.54	2.56 ± 0.19	25.36

Table 2

Analgesic Activity

Compound	Abdominal constriction		Trunk twisting		Extention of hind limbs	
	Mean ± SEM	% red	Mean ± SEM	% red	Mean ± SEM	% red
control	30.1 ± 0.11	NA	18.4 ± 0.41	NA	11.6 ± 0.07	NA
standard	14 ± 0.13	53.48	7.11 ± 0.02	61.35	5.18 ± 0.01	55.34
II_a	28.96 ± 0.13	3.78	16.79 ± 0.14	8.75	10.28 ± 0.17	11.37
II_b	21.7 ± 0.15	27.9	11.3 ± 0.08	38.58	9.10 ± 0.81	21.55
II_c	22.03 ± 0.01	26.81	14.15 ± 0.12	23.09	8.26 ± 0.02	28.79
II_d	27.50 ± 0.11	8.63	17.04 ± 0.11	7.39	11.11 ± 0.13	4.22
II_e	25.37 ± 0.16	15.71	16.17 ± 0.11	12.11	9.71 ± 0.03	16.29

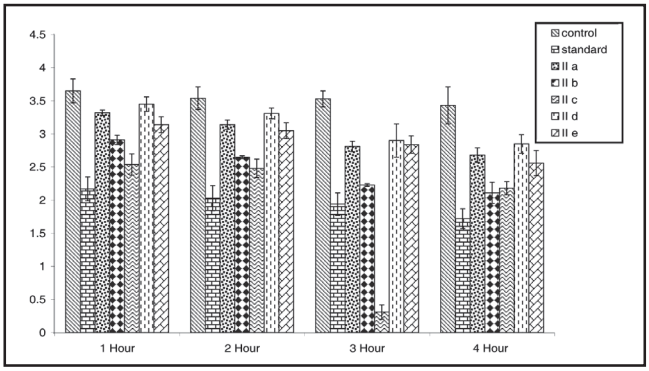


Fig.1: ANTI INFLAMMATORY ACTIVITY: Graphical representation of percentage inhibition of compounds IIa,IIb, IIc, IId, V and standard drug in Carrageenen induced paw-oedema method

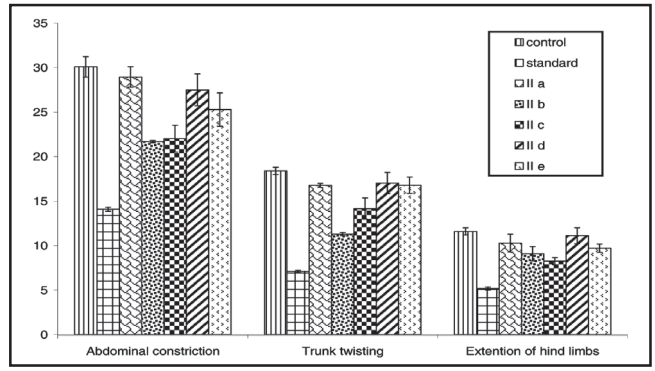


Fig.2: Analgesic Activity Graphical representation of percentage inhibition of compounds IIa,IIb, IIc,IId,V and standard drug in actic acid-induced writhing test on mice

Analgesic Activity

Acetic acid Induced writhing method

The results of the aceticacid induced writhing was represented in table-2 & figure-2. The % inhibitions were 11.37%,21.55%, 28.79%,4.29%,16.29% respectively for compound IIa, compound IIb,compound IIc, compound IId and compound V respectively with significance value less than 0.05. Compounds IIb &IIc shows moderate activity compared standard drug phenylbutazone (55.34%) where as compound IId shows weak activity against standard with % inhibition of 4.22% .

Acknowledgement

The authors are thankful to the management of Bharat institute of pharmacy for providing the required facilities to carryout the Research work.

References

[1]. Ferrarini, P. L.; Mori, C.; Tellini, N. *Farm. Ed. Sci.* 1990, *45*, 385.

[2]. 2. Lirvinov, V. P. *Adv. Heterocycl. Chem.* 2006, *91*, 222.

[3]. Helmut, H.; Juergen, P.; Hans, Z.; Bruno, W.; Otto, K. W. *Ger. Offen*. 3907938, 1990; *Chem.Abstr*. 1991, *114*, 122342f.

[4]. Muller, C. J.; Grahner, B.; Heber, D. *Pharmazie* 1994, *49*, 878.

[5]. Ferrarini, P. L.; Badawneh, M.; Franconi, F.; Manera, C.; Miceli, M.; Mori, C.; Saccomanni,G. *Farmaco* 2001, *56*, 311.

[6]. Badawneh, M.; Ferrarini, P. L.; Calderone, V.; Manera, C.; Martinotti, E.; Mori, C.;Saccomanni, G.; Testai, L. *Eur. J. Med. Chem*. 2001, *36*, 925.

[7]. Ferrarini, P. L.; Manera, C.; Mori, C.; Badawneh, M.; Saccomanni, G. *Farmaco.* 1998, *53*,741.

[8]. G. Abbiati, A. Arcadi, F. Martinelli, and E. Rossi, Synthesis, 2002, 1912.

[9]. T. Sakamoto, Y. Kondo, and H. Yamanaka, Chem. Pharm. Bull., 1985, 33, 4764.

[10]. F. W. Hartner, Y. Hsiao, K. K. Eng, N. I. Rivera, M. Paluki, L. Tan, N. Yasuda, D. L. Hughes, S.Weissman, D. Zewge, T. King, D. Tschaen, and R. P. Volante, J. Org. Chem., 2004, 69, 8723.

[11]. J. Bernstein, B. Stearns, E. Shaw, and W. A. Lott, J. Am. Chem. Soc., 1947, 69, 1151.

[12]. K. Mogilaiah and R. B. Rao, Synth. Commun., 2003, 32, 747.

[13]. J. J. Baldwin, E. L. Engelhardt, R. Hirschmann, G. S. Ponticello, J. G. Atkinson, B. K.Wasson, C. S.Sweet, and A. Scriabine, J. Med. Chem., 1980, 23, 65.

[14]. F. P. Mazza and C. Migliardi, Atti Accad. Sci. Torino, Classe Sci. fis., Mat. Nat., 1940, 75, 438;Chem. Abstr., 1942, 36, 5477.

[15]. T. Higashino, K. Suzuki, and E. Hayashi, Chem. Pharm. Bull., 1978, 26, 3242.

❖

ISSN 0973 – 9874

Dentritic Nanocomposite for the Delivery of some Anti-HIV Bioactive

RAM KUMAR CHOUDHARY*[1], VIJAYARAJ KUMAR P*[2] AND JAYAVEERA KN[3]

1. Department of Pharmaceutical sciences, Jawaharlal nehru technological university, Anantapur,India(AP).
2. Faculty of pharmaceutical sciences, UCSI(University College Sadaya International)University, School of Pharmacy, No.1 Jalan Menara Gading, 56000 Cheras, Kuala Lumpur, Malaysia.
3. Head of chemistry Department, Jawaharlal Nehru Technological University, Anantapur, India(AP).

ABSTRACT

The present study was aimed at developing and exploring the use of PEGylated citric acid dendritic architecture for the delivery of an anti-viral drug, Nevirapine. Nevirapine falls in the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretroviral. Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs bind allosterically at a distinct site away from the active site termed the NNRTI pocket. For this study, PEGylated citric acid dendritic architecture was synthesized and loaded with Nevirapine. Further, analytical parameter like IR, NMR was confirming the structure drug entrapment. The PEGylation of the systems was found to have increased their drug-loading capacity, reduced their drug release rate. The systems were found suitable for sustained delivery of Nevirapine.

KEYWORDS: Dendrimer, Nanoparticle, Nevirapine and Drug Loading.

Introduction

Dendrimers are a new class of polymeric materials. The term Dendrimer was derived from Greek word Dendron (tree) and meros (part) and relates to the symmetrical branch-like structure of the polymers. They are highly branched, monodisperse macromolecules that can be sub divided into three architectural components: a central core, branched cell, interior branch cells possessing surface groups [1].

Poly ethylene glycol (PEG) is typically a clear, colorless, odorless substance that is soluble in water, stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm, as reviewed earlier [2, 3]. It has been shown that covalent attachment of poly(ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time [4,5].

Moreover, a number of studies have demonstrated that poly (ethylene glycol) chains grafted to surface of polymer micelles and liposomes suppress their interaction with plasma proteins and cells and prolong their blood elimination half-life [6-11]. On the basis of these findings, it seems that dendrimers covered with poly(ethylene glycol) grafts are attractive compounds as drug carriers in *in vivo*. Such molecules are expected to encapsulate drugs in their dendrimer moiety and reveal biocompatibility due to their hydrophilic shell consisting of poly (ethylene glycol) grafts [12-15].

The technology of polyethylene glycol conjugation refers to the conjugation of drug or drug moiety to polyethylene glycol (PEG) through the covalent or non-covalent interaction. The technology is also called PEGylation [16]. PEG can be attached to the dendrimer to 'disguise' it and prevent the body's defense mechanisms from detecting it, thereby slowing the process of breakdown. This allows the delivery system to circulate in the body for an extended time period, maximizing the opportunities for the drug to reach the relevant sites [17].

The present studies were aimed at developing and exploring the efficiency of Pegylated citric acid dendritic nanostructure for solubilization and sustain release of an anti-viral drug. Nevirapine was selected for incorporation

*Address for correspondence:
e-mail: ram.chy6@gmail.com

into pegylated citric acid dendritic nanostructure based on its antiviral activity and its hydrophobic nature.

Experimental

Materials

Nevirapine was a gift sample from hetero drugs, India. Poly Ethylene Glycol (400, 4000) diacid (acid number 175, 96-98% from fluka), Citric Acid (Merk), Pyridine (Merk), Thionyl chloride ($SOCl_2$-Merk), Potassium Permanganate ($KMNO_4$), Sulphuric Acid(H_2SO_4), N,N' Dicyclohexyl Carbodimide(DCC) [18].

Synthesis of 3rd generation citric acid dendrimer

Novel citric acid dendritic nanostructures were synthesized by previously reported method (Hassan Namazi and Mohsen Adeli 2003) with slight modification [19]. 0.175 moles of polyethylene glycol (PEG) 400 was weighed accurately and transferred into a round bottom flask. 3.75g of sodium carbonate (Na_2CO_3) was dissolved in 38 ml of water and 35.5g of Potassium permanganate ($KMnO_4$) was dissolved in 694ml of water. Both the solutions were mixed and added to the round bottom flask containing polyethylene glycol. The mixture was stirred vigorously on magnetic stirrer for 3-4hours, and then cooled to 4-5°C by immersion in an ice bath. The reaction mixture was allowed to attain room temperature. The precipitated manganese dioxide was removed by filtration. The obtained filtrate was cooled and heated continuously to get a concentrate filtrate of about 100ml. The solution of filtrate was cooled and was covered with a layer of ether. The solution was kept aside for the separation of ether and aqueous layer. The extraction of aqueous layer was done by two or three portions of ether. The collected aqueous layer was heated on a water bath for removal of ether. The precipitated polyethylene glycol diacid (G1) was filtered. 0.1mol Polyethylene glycol diacid and 0.3mol thionylchloride are placed in a round bottom flask equipped with a magnetic stirrer and a condenser with drying tube. The reaction mixture is stirred and heated in a 70°C. Thus obtained chlorinated PEG (ClOC-PEG-COCl) (G 1.5) was further purified by using column chromatography. 0.1 mol of G 1.5 dendrimer was dissolved completely in dimethyl formide (DMF) kept in an ice bath. 0.2 mol of citric acid was dissolved in DMF completely, to this solution 3 moles of pyridine was added drop wise. It was stirred and was kept aside in an ice bath for 24 hour. After 24 hours it was taken and both the solutions was mixed and kept in incubator for 6hrs at 55-60°C. Thus obtained 2nd G citric acid dendrimer was further purified by using column chromatography. Citric acid dendrimers up to 3.0G were prepared by repetition of all the above steps consecutively, with increasing quantity of citric acid and thionylchloride as shown in Scheme-I. IR spectroscopy was carried out using Perkin-Elmer IR spectrophotometer.

Synthesis of pegylated citric acid dendrimer

Step 1: 5moles of N,N' Cyclohexyl carbodimide (DCC) weighed accurately and added to 1mol of Citric acid Dendrimer by dissolving in (10ml) Di methyl formamide (DMF) completely, and the mixture stood for 4 days at 40°C.

Step 2: From the above formed residue was weighed and added to PEG 4000 (5 mol) by dissolved completely in chloroform (150ml), are set to reflux for 2-3 days for esterification. The obtained mixture was filtered by placing in the separating funnel when mixed with water (5ml), so as to remove N,N' Cyclohexyl carbodimide (DCC). Until the formation of final product dry the mixture.

Drug loading in formulations

The Pegylated citric acid dendrimers so synthesized were dissolved in Dimethyl Formamide (DMF) and mixed with 100 molar times of Nevirapine and allowed the Nevirapine to dissolve. The mixed solution was allowed to incubate with slow magnetic stirring (50 rpm) using teflon beads for 45 hr. This solution was twice dialyzed by using Cellulose dialysis bag (MWCO 12-14 KDa) under strict sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically (λ_{max} 290 nm) to determine indirectly the amount of drug encapsulated with the system. The dialyzed formulations were dried and used for further characterization.

Morphology of dendrimers

Digital electron microscopy (EM) was performed to investigate particle size and provide information on nanoparticle morphology. Prepared and dialyzed NVP loaded PEGylated dendrimer formulations were used for image analysis Digital electron microscopic studies.

Results and Discussion

Syntheses of 3rd generation pegylated citric acid dendrimer:

3rd generation Pegylated citric acid dendrimer were synthesized by the procedure reported by Hassan Namazi and Mohsen Adeli 2003 [19]. IR data proved the synthesis. IR spectrum (KBr); 1112.93 cm^{-1} for C-O; 1242.16 cm^{-1} for CH_2 rock; 1467.83 cm^{-1} for CH_2 scissor; 2885.51cm^{-1} for CH_2 asymmetric stretching; the peaks are disappeared for COOH stretch at 3392 cm^{-1}; the CO2 stretching at 1780 cm^{-1}, and C=O stretching peaks at 2360 cm^{-1} were merged due to pegylation of dendrimer. The above data confirmed that the compound was PEGylated citric dendrimer of 3rd generation.

Table A.1
Selected values of IR Spectroscopic Data (KBr) for Dendrimers

S.No	Functional Group	Observed Value (cm^{-1})	Theoretical value (cm^{-1})
1	C-O	1112.93 cm^{-1}	900-1300
2	CH_2 _rock	1242.16 cm^{-1}	1300-1500
3	CH_2 scissor	1467.83 cm^{-1}	1300-1500

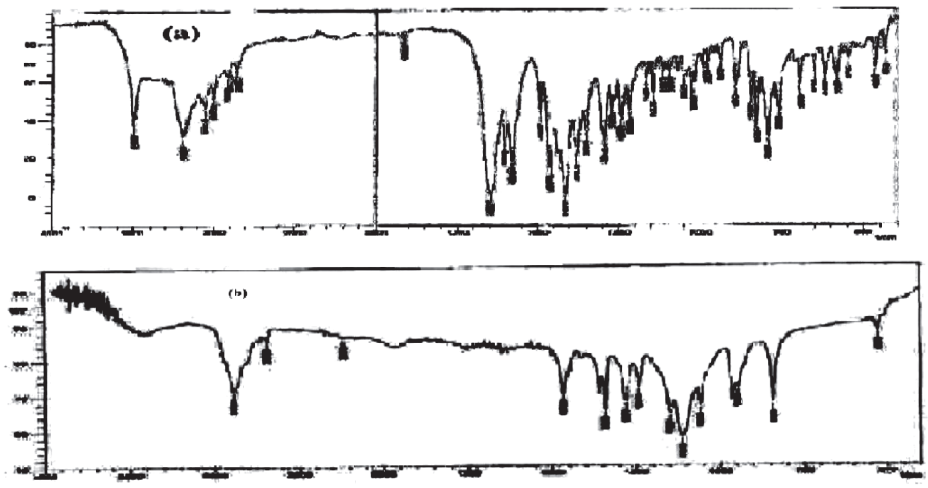
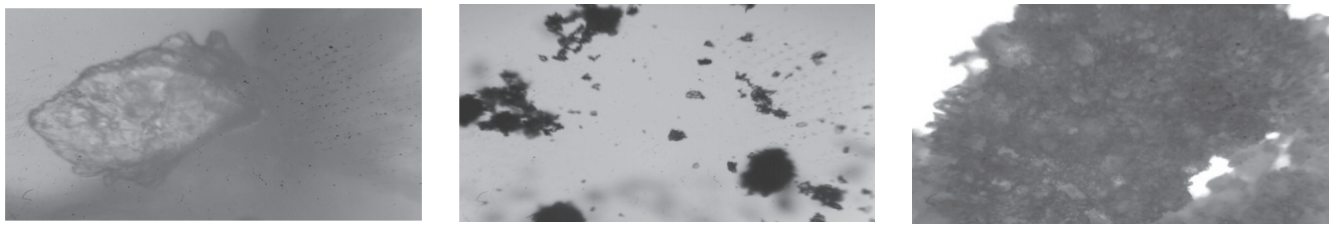


Fig.1a, 2 b. IR spectrum of Nevirapine and Pegylated Citric acid dendrimer.



(a) **(b)** **(c)**

Fig.3: a,b and c show that the drug loaded PEGylated dendrimers.

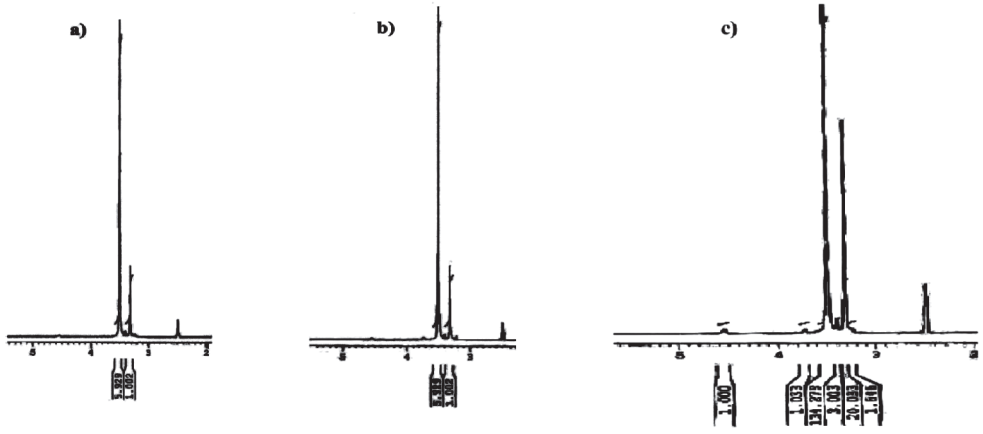


Fig. 4: NMR analysis of (a) Nevirapine (b) Pegylated Citric acid dendrimer (c) Drug Loaded Pegylated Citric acid dendrimer.

Morphology of dendrimers

Digital Electron Microscopes micrographs (Fig. **3. a,b** and **c**) show that the drug loaded PEGylated dendrimers.

Nuclear Magnetic Resonance (NMR)

The NMR peaks of Nevirapine-3G PEGylated Citric acid Dendrimer complex as compared to plain Nevirapine and 3G PEGylated Citric acid Dendrimer confirms the drug loading. The characteristic peaks of Nevirapine and 3G PEGylated Citric acid Dendrimer are present in Nevirapine loaded 3G PEGylated Citric acid Dendrimer complex. (Fig.4)

Conclusion

The present work establishes suitability of novel pegylated citric acid dendritic nanostructure as drugs sustained delivery system for nevirapine. From the present study, it can be concluded that the pegylated citric acid dendritic nanostructure systems can act as long circulatory sustained drug delivery.

References:

[1]. Tomalia DA, Baker H, Dewald JR, Hall M, Kallos G, Martin S, Roeck J, Ryder J & Smith P et al. A new class of polymers: Starburst-dendritic macromolecules. Polymer J 1985; 17: 117-132.

[2]. Bhadra, D.; Bhadra, S.; Jain, P.; Jain, N.K. Pharmazie, 2002, 57, 5.

[3]. Bhadra, D.; Bhadra, S.; Jain, N.KJ. Drug Del. Sci. Tech., 2005, 15,65.

[4]. Abuchowski, A.; Palczuk, N.C.; Van Es, T.; Davis, F.F. J. Biol.Chem., 1977, 252, 3578.

[5]. Abuchowski, A.; McCoy, J.R.; Palczuk, N.C.; van Es, T.; Devis, F.F. J. Bio. Chem., 1977, 252, 3582.

[6]. Yokoyama, M.; Okano, T.; Sakurai, Y.; Ekimoto, H.; Shibazaki, C.; Kataoka, K. Cancer Res., 1991, 51, 3229.

[7]. Klibanov, A.; Maruyama, K.; Torchilin, V.P.; Huang, L. FEBS Lett., 1990, 268, 235.

[8]. Blume, b.; Cevc, G. Biochim. Biophys. Acta, 1990, 1029, 91.

[9]. Woodle, M.C.; Lasic, D.D. BioChim. Biophys. Acta, 1992, 1113,171.

[10]. Lasic, D.D.; Needham, D. Chem. Rev., 1995, 95, 2601.

[11]. Margerum, L.D.; Campion, B.K.; Koo, M.; Shargill, N.; Lai, J.J.; Marumoto, A.; Sontum, P.C. J. Alloys Compd., 1997, 249, 185.

[12]. Kojima, C.; Kono, K.; Mayuyama, K.; Takagishi, T. Bioconjugate Chem., 2000, 11, 910.

[13]. Liu, M.; Kono, K.; Fréchet, J.M.J. J.Control. Release, 2000, 65,121.

[14]. Liu, M.; Kono, K.; Fréchet, J.M.J. J. Polym. Sci. Part A Polym.Chem. Ed., 1999, 37, 3492.

[15]. Ambade, A.V.; Savariar, E.N.; Thayumanavan, S. Mol. Pharm.,2005, 264.

[16]. http://www.esciencenews.com/articles/2010/03/11/pegylated.dendrimers.a.novel.mechanism.drug.delivery

[17]. Advanced PEGylation for the development of raloxifene hydrochloride, BCS class II drug JJ Bandela, CH Anupama Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam (Women' University), Tirupati (A.P) - 517 502, India

[18]. Hand book of Pharmaceuticals Excipients, 5th edition, edited by Raymond e Rowe, Paul J Sheskey and Sian C Owen: London, 2006: published by pharmaceutical press & American pharmacist Association.

[19]. Namazi H and Adeli M, Novel linear–globular thermoreversible hydrogel ABA type copolymers from dendritic citric acid as the A blocks and poly(ethyleneglycol) as the B block. European Polymer Journal, 2003;39:1491-1500.

ISSN 0973 – 9874

Design, Optimization and Characterization of Glipizide Sustained Released Matrix Tablet

PANDA NIRANJAN[1*], PANDA K.C[1], DAS B.B[1] AND VENKATESHWAR REDDY A[1]

1.Anwarul Uloom College of Pharmacy, Newmallepally, Hyderabad-500001, A.P, India.

ABSTRACT

The aim of the present study was to optimize and characterize sustained released Glipizide matrix tablet using hydrophilic synthetic polymers like hydroxyl propyl methyl cellulose (HPMC) and hydrophobic polymer like ethyl cellulose (EC). Matrix tablet of Glipizide was prepared by wet granulation method at different ratios of HPMC and EC to optimize the release rate for once daily medication. In-vitro release study was performed by using USP type-II three station dissolution apparatus. Further more in-vitro data of optimized sustained release Glipizide tablets was compared with conventional marketed SR tablets (Glipizide, India). FTIR study was carried out to ensure drug polymer compatible study. Other precompression and postcompression characterization of tablet was carried out and the results are satisfactory according to the pharmacopoeia specifications. Accelerated stability studies were carried out to conform the stability of dosage forms.

Key words: Matrix tablets, Glipizide, HPMC, EC, Sustained release.

Introduction

To reduce the frequency of administration and to improve patient compliance for drugs having shorter half life, attention has been made to formulate sustained release (SR) or controlled release (CR) drug delivery system **[1]**. Sustained or controlled release delivery systems can achieve predictable and reproducible release rates, extended duration of activity for drugs having shorter half life, decrease toxicity and reduction required dose for optimization of therapy and better patient compliance **[2, 3]** . Matrix type sustained delivery systems are popular as dosage forms because of their dose uniformity, ease of manufacturing, ease of transportation and cost effectiveness. The release rate can be controlled by using different type and proportion of polymers. Hydrophilic polymer (HPMC, Sodium Alginate, Natural Gums) matrix system are widely used for designing oral sustained release delivery system because of their flexibility to provide a desirable drug release profile, cost effective and broad acceptance in the gastro intestinal tract at any biological P^H and provide good bioavailability of the active ingredients. But the use of hydrophilic polymer alone causes rapid release of drug and has uncontrolled rate of hydration in GIT. To overcome this problem, hydrophobic polymers are included in the matrix system. Keeping this in view, the present investigation is aimed at designing suitable release matrix tablet using polymers like HPMC K15M, EC and poly vinyl pyrrolidone (PVP) **[4, 5]**.

Glipizide is a second generation sulfonyl urea compound that is used as an oral hypoglycemic agent and is commonly prescribed for the treatment of patient with type-II diabetes **[6]**. it is used adjunct to diet for the management of non insulin dependent diabetes mellitus in patients whose hyperglycemia can't be controlled diet and exercise alone. Glipizide stimulate insulin secretion from the cells of pancreatic islets tissue, increases the concentration of insulin in pancreatic vein and may increase the number of insulin receptors **[7]**. Glipizide is a weak acid (P^{Ka}=5.9), practically insoluble in water and in acidic environment and highly permeable (BCS class-II) drug **[8]**. The oral absorption is uniform, rapid and complete with a bioavailability of nearly 90% and an elimination half life of 2-4 hrs. Glipizide is reported to have short biological half life requiring it to be administered in 2 to 3 dosages of 2.5 to 10 mg per day **[9]**. SR formulations that would maintain plasma levels of drugs for 8 to 12 hrs might be sufficient for once a day dosing for Glipizide. Chemically it is a cyclohexyl sulfonyl urea analogue and is effective in treating patients who show resistant to all other oral hypoglycemic agents.

Material and Methods

Materials

Glipizide BP was procured as gift sample from Sunshine

*Address for correspondence
Email: niranjanpharma82@gmail.com

lab. Ltd. with batch no. 010707/GP, assay-99.4%, LOD-0.14% w/w. HPMC K15M, EC, PVP were purchased from SD fine chemicals, Mumbai. All other solvents and reagents were of analytical grade.

Methods

Formulation of matrix tablet:

Matrix tablets of Glipizide were prepared by wet granulation method. Accurate quantities of all ingredients were weighed and passed through sieve no #80 before their use in formulations. For each formulation specific and accurate quantities of powder like Glipizide, HPMC, EC, PVP, and lactose were blended uniformly and passed through #20. Acacia mucilage was used as binder. The aggregates formed after addition of binder were initially dried 5-10 minutes to reduce moisture level and to prevent sticking with sieve. The aggregates were passed through sieve # 20 to get granules. The granules are dried at 40° C for 20 minutes to reduce moisture content upto 2-5 %. Magnesium stearate and talc were used as lubricants and the required quantities are mixed with dried granules for 2-3 minutes **[10]**. After lubrication the formulations were evaluated for angle of repose, bulk density, compressibility; prior to compression. The formulas for different formulations are given in table-1 and same method was followed for all the formulations.

Evaluation of Glipizide granules [11, 12, 13]:

Angle of repose

The angle of repose of prepared Glipizide granules were evaluated by simple funnel method. The accurately weighed granules were taken in a funnel. The height of the funnel was maintained approximately 2cm from the top of powder pile. The granules were allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and the angle of repose was calculated using the following equation.

$$\tan \alpha = H/R$$

Where H and R are the height and the radius of the powder cone respectively. The angles of repose of different formulations are given in table-2.

Bulk density

Both the loose bulk density (LBD) and tapped bulk density (TBD) were determined. The quantity of 2 gm of powder from each formula, previously lightly shaken to break any agglomerates formed; was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight on to a hard surface form the height of 2.5 cm at second interval. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulae. The results of each formulation were given in table-2.

$$LBD = \frac{\text{weight of the powder}}{\text{volume of the packing}}$$

$$TBD = \frac{\text{weight of the powder}}{\text{tapped volume of the packing}}$$

Compressibility index (Carr's index)

The compressibility index of the granules was determined by Carr's compressibility index by following formula. The results of each formulation were given in table-2.

$$\text{Carr's index (\%)} = \frac{TBD - LBD}{TBD} \times 100$$

Hausner's ratio

The Hausner's ratio of prepared Glipizide granules was determined by following formula. The results of each formulation were given in table-2.

Table 1

Formulations of Glipizide Matrix Tablets

F. No.	Glipizide (mg)	HPMC (mg)	EC (mg)	PVP (mg)	Lactose (mg)	Acacia (mg)	Mg. stearate (mg)	Talc (mg)	Total wt. (mg)
F_1	10	40	-	5	120	20	3	2	200
F_2	10	50	-	5	110	20	3	2	200
F_3	10	60	-	5	100	20	3	2	200
F_4	10	70	-	5	90	20	3	2	200
F_5	10	20	25	5	115	20	3	2	200
F_6	10	25	25	-	115	20	3	2	200
F_7	10	30	25	-	110	20	3	2	200
F_8	10	30	20	5	110	20	3	2	200
F_9	10	30	30	5	100	20	3	2	200
F_{10}	10	20	30	5	110	20	3	2	200

$$Hausner's\ ratio = \frac{TBD}{LBD}$$

Total Porosity

Total porosity was determined by measuring the volume occupied by a selected weight of a powder (V_{bulk}) and true volume of the granule (V: the space occupied by the powder exclusive of spaces greater than the inter molecular space). The results of each formulation were given in table-2.

$$Porosity\ (\%) = \frac{vVbulk - V}{V}\ x\ 100$$

Drug content

An accurately weighted amount of powdered Glipizide granules (100 mg) was taken and dissolved in 50 ml of methanol with gentle heating on a water bath, cooled and filtered. Then sufficient amount of methanol was added to produce 100 ml. from that filtrate 5ml was withdrawn and diluted upto 50 ml with methanol. The absorbance was measured spectrophotometrically at 274 nm after suitable dilution. The results of each formulation were given in table-2.

Evaluation of prepared Glipizide matrix tablet:

Thickness

The thickness of tablet was determined by using veneerss calipers. 10 tablets from each batch were used and average values were calculated. The results of each formulation were given in table-3.

Hardness

It is the tensile strength of tablets expressed in kg/cm^2 was determined by using the Monsanto hardness tester. It is the pressure required to break the tablet into two halves. The results of each formulation were given in table-3.

Weight variation test

Weight variation test was done with 20 tablets from each formulation. It is the individual variation of tablet weight from the average weight of 20 tablets. Not more than 2 of the individual weights may deviate from the average weight by more than the % deviation and none should deviate by more than twice that of the % (limit for 130 to 324 mg is NMT 7.5%).The results of each formulation were given in table-3.

Friability

This test is a method to determine physical strength of uncoated tablets upon exposure to mechanical shock and attrition. Pre weighed samples of tablets were placed in the friabilator (Roche friabilator) and operated for 100 revolutions (25 rpm speed). Tablets were dusted and reweighed. The test complies if tablets not loose more than 1% of their weight. The results of each formulation were given in table-3.

In-vitro dissolution study

The In-vitro dissolution studies were performed using USP apparatus type-II (dissolution rate test three stage apparatus, Intelli series) at 50 rpm. The dissolution medium (900 ml) consisted of 0.1N HCl for first 2 hrs and phosphate buffer P^H 7.4 from 2-12 hrs, maintained at temperature of 37 $\pm$ 0.5 ^{o}C. The drug release at different time interval was measured by UV-Visible spectrophotometer at 274 nm (ELICO SL 164 double beam UV-Visible spectrophotometer). The results of each formulation were given in table-4.

Kinetic release profile [15, 16]

To study the release kinetics, data obtained from in vitro drug release studies were plotted in various kinetic models. Zero order kinetic model was plotted as cumulative percentage of drug release vs. time.

Table 2

Physical Properties of Granules (Evaluation of Glipizide Granules)

F. No.	Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of repose	Total porosity (%)	Compressibility (%)	Hausner's ratio	Drug Content(%)
F_1	0.438	0.524	23.48	19.65	16.41	1.20	95.39
F_2	0.492	0.593	22.59	25.58	17.03	1.21	96.73
F_3	0.468	0.508	22.98	8.53	7.87	1.08	94.48
F_4	0.505	0.583	23.67	15.69	13.55	1.16	98.71
F_5	0.408	0.496	24.87	26.58	17.74	1.22	99.07
F_6	0.396	0.492	23.68	29.20	19.51	1.24	98.45
F_7	0.487	0.526	24.59	8.00	7.38	1.08	97.13
F_8	0.542	0.598	23.61	10.35	9.36	1.10	98.40
F_9	0.473	0.529	24.05	11.85	10.58	1.12	97.89
F_{10}	0.479	0.521	23.13	8.81	8.06	1.09	96.65

$C = K_0 t$ where C = Cumulative drug release at time t.
K_0 = Zero order rate constant

First order kinetic model was plotted as log cumulative percentage of drug remaining to be released vs. time.

$$log\ C = log\ C_0 - \frac{Kt}{2.303}$$

where C = Cumulative drug release at time t.
C_O = initial drug concentration
K = first order rate constant

Higuchi kinetic model was plotted as cumulative percentage of drug release vs. square root of time.

$Q = K\ t^{1/2}$

Q = Cumulative percentage drug release at time t.

Drug excipient compatibility study [17]

The excipient interaction was studied by comparing the FTIR spectrum of the optimized formulation (F_8) with that of spectrum of Glipizide pure drug and excipients (HPMC, EC, and PVP). The peaks that appear in the formulation F_8 are also clearly seen in Glipizide pure drug. Thus the comparison showed that there is no drug interaction between the drug and other excipients of formulation such as HPMC, EC, PVP, lactose, magnesium stearate, talc. The FTIR studies were carried out at SIPRA LAB., Hyderabad, India. The peaks of Glipizide pure drug and excipients (HPMC, EC, and PVP) are given in figures 1-5.

Stability studies (as per ICH guidelines) [18]

The optimized formulation was evaluated for stability studies as per ICH guidelines. The formulation was exposed to conditions with different temperature and humidity like 30 °C ± 2 °C/ 65% ± 5% RH and 40 °C ± 2 °C/ 75% ± 5% RH for 90 days. Former condition is for room temperature and later is for accelerated condition. After that period the product was evaluated for friability, hardness, weight variation, thickness, drug content and in vitro release study.

Results

Results of Glipizide granules (physical properties of granules)

The prepared granules of Glipizide was evaluated on different physical parameters like instant angle of repose, LBD, TBD, compressibility index, total porosity and drug content which are given in table-2. The result of angle of

Table 3
Physical Properties of tablets
(Evaluation of Glipizide tablets)

F. No.	Average hardness (kg/cm²)	Average Weight Variation(%)	Average friability (% w/w)	Average thickness (mm)
F_1	4.97	4.381	0.69	4.89
F_2	4.54	3.912	0.54	4.90
F_3	4.89	3.124	0.62	4.89
F_4	4.95	3.120	0.45	4.82
F_5	5.02	2.981	0.51	4.84
F_6	5.01	2.431	0.38	4.82
F_7	5.00	3.012	0.43	4.84
F_8	4.91	2.861	0.48	4.83
F_9	4.98	2.753	0.49	4.85
F_{10}	4.97	2.542	0.40	4.84

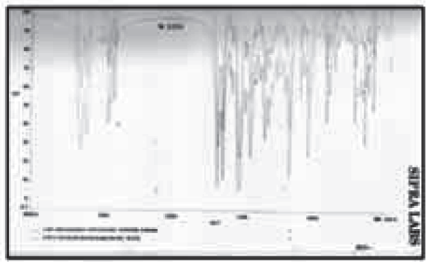


Fig.1: FTIR Spectra of Glipizide pure drug

Fig.2: FTIR Spectra of HPMC

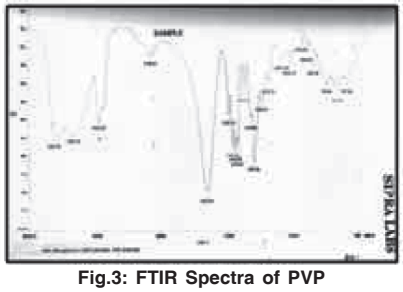


Fig.3: FTIR Spectra of PVP

Fig.4: FTIR Spectra of Ethyl cellulose

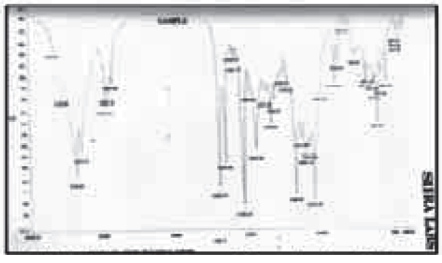


Fig.5: FTIR Spectra of formulation F8

Fig.1-5: I.R. Glipizide pure drug and excipients (HPMC, EC, and PVP)

Table 4

In-vitro drug released studies (cummulative % of drug release)

Times(hr)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
1	10.49	8.4	6.98	6.12	8.14	5.48	6.41	7.48	4.81	3.74
2	25.84	22.61	18.45	17.59	14.39	10.93	9.98	10.19	7.42	5.79
3	42.39	38.76	33.72	31.48	23.48	15.48	17.64	15.65	12.54	12.35
4	65.27	60.45	49.48	46.72	39.49	20.24	29.46	26.59	23.19	23.48
5	82.44	78.97	62.87	59.4	48.48	41.43	41.65	37.41	31.45	34.87
6	97.48	91.45	86.29	74.61	64.59	53.48	53.48	48.19	42.14	40.49
7	99.52	98.43	96.45	86.53	77.72	69.91	68.71	61.45	54.97	51.94
8	99.54	98.45	99.12	96.45	87.91	84.52	81.68	70.89	65.19	64.89
9	99.34	98.35	99.11	96.48	96.48	97.38	89.97	81.19	78.9	73.84
10	99.14	98.42	99.14	96.45	99.4	97.39	97.48	88.45	89.45	84.9
11	99.43	98.46	99.13	96.47	99.41	97.36	98.97	92.78	94.26	90.95
12	99.41	98.41	99.12	96.48	99.42	97.38	98.95	98.46	96.34	95.43

repose ranged from 22.59 to 24.87 and % compressibility index ranged from 7.03 to 19.51. The result of LBD ranged from 0.408 to 0.542 and TBD ranged from 0.492 to 0.598. The results of % porosity of the granules ranged from 8.53 to 24.20. The drug content in a weighed amount granules of all formulations ranged from 94.48% to 99.07%. The Hausner's ratio ranged from 1.08 to 1.24.

Results of Glipizide tablets (physical properties of tablets)

The thickness of the prepared tablets ranged from 4.82 to 4.90 mm. The average % weight variations of 20 tablets of each formulation were less than ± 5%. The hardness of the tablets of all batches ranged from 4.54 to 5.02 kg/cm^2 and the percentage friability ranged from 0.38 to 0.69 % w/w. the results are given in table-3.

In-vitro release study

The results of cumulative percentage release of the drug after specified interval of times is given in table-4.

In-vitro kinetic study

In-vitro released data for optimized formulation (F_8) was tested for zero order, first order and Higuchi model. The slope value and regression coefficient was calculated for each model and given table-5.

Stability study

Various physical parameters of tablets like friability, hardness, weight variation, thickness, drug content and in-vitro released study of optimized fresh formulation (F_8) and after accelerated stability study were compared. The test complies with stability conditions.

Discussion

Glipizide is an oral hypoglycemic agent which is a commonly prescribed drug for treatment of patients with type-II diabetes (non insulin dependent). Diabetes mellitus is chronic disease and requires constant level of insulin that can be achieved by constant and uniform supply of drug for extended period of time.

The granules for tablet are prepared by wet granulation method which is a conventional method and most advantageous than others. A granule is an aggregation of component particles that is held together by the presence of bonds of finite strength. Physical properties of granules such as specific surface area, shape, hardness and size can significantly affect rate of dissolution of drugs and hence overall bioavailability of drug in heterogeneous formulation. The result of angle of repose is less than 30^o for all formulations that indicates good flow properties of granules. Compressibility index is also less than 16% for most of the formulations except F_2, F_5 and F_6 which indicates good to excellent flow properties. Bulk density of granules with formulations F_8 and F_4 are higher than others which indicate presence of more fines in the formulations. The percentage porosity values of the granules indicating that the packing of the granules may range from close to loose packing.

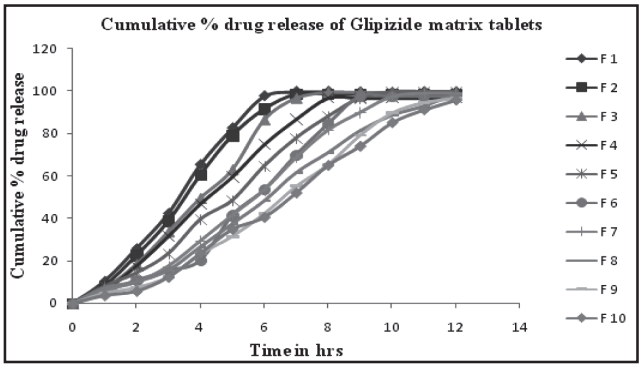


Fig.9 : Graph of in-vitro release study of formulation F1 - F10

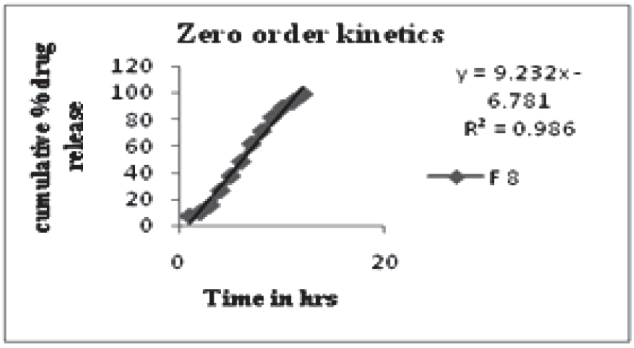


Fig.6: Graph of Zero order release kinetics

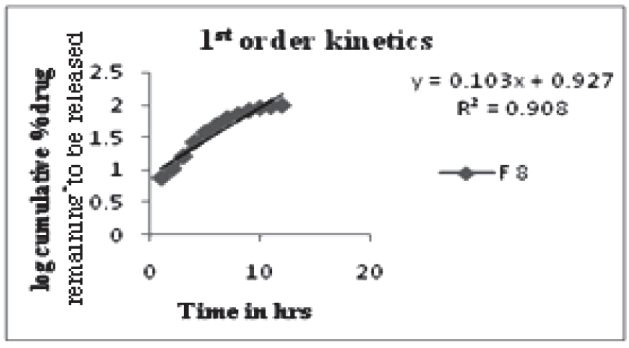


Fig.7: Graph of first order release kinetics

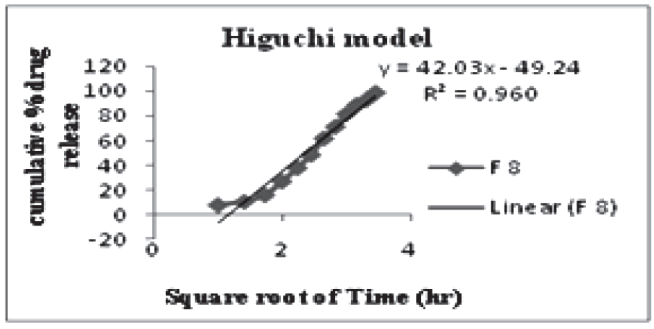


Fig.8: Graph of Higuchi Kinetic Model

Table 5
In-vitro release kinetic study

Formulation	Regression coefficient of Zero order	Regression coefficient of 1st order	Regression coefficient of Higuchi model	Conclusion
Optimized Glipizide SR Tablet (F_8)	0.986	0.908	0.960	Release kinetics Followed zero order

Table 6
Comparison of dissolution data of stability study at accelerated conditions of optimized batch (F_8)

Time (hr)	Initial	After 15 days	After 30 days	After 45 days	After 90 days
1	7.48	7.10	6.48	5.87	4.98
2	10.19	10.59	8.65	7.77	6.44
3	15.65	14.68	12.81	11.57	10.54
4	26.59	24.32	21.45	20.39	19.56
5	37.41	35.63	32.18	29.67	28.45
6	48.19	47.38	45.51	42.40	39.85
7	61.45	60.57	56.48	55.63	52.41
8	70.89	68.42	66.67	64.97	61.48
9	81.19	79.59	75.62	72.47	69.59
10	88.45	85.48	82.48	80.69	78.70
11	92.78	91.36	87.12	84.08	81.63
12	98.46	97.29	92.28	91.79	90.45

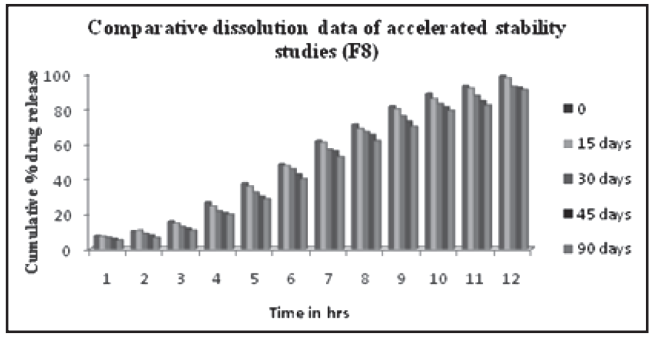


Fig.10: Histogram showing dissolution data of Accelerated stability studies

The physical properties like average hardness, average weight variation, average friability, thickness of tablets were studied. The friability of most of the formulations is within acceptable range. The formulation F_1, F_2 and F_3 having higher friability may be due to use of ethanol as a granulating solvent alone because ethanol alone could not provide sufficient binding to granules. Hardness, weight variation

and thickness are within acceptable range from batch to batch. The in-vitro drug release characteristics were studied in simulated gastric and intestinal fluids for a period of 12 to 14 hrs using USP type-II dissolution apparatus. Formulation F_1 to F_4 release the drug within 6-8 hrs may be due to HPMC as control released polymer in the formulation alone.

So it required to use EC in the formulation. The formulation F_5 to F_{10} release the drug from 10 to 12 hrs. Different proportion of HPMC and EC are used to optimize release characteristics. The formulation F_8 was found to release the drug in a constant and predetermined rate upto 12 hrs, in this formulation initial release is more which is necessary for loading dose to elicit pharmacological response. Drug-excipients compatibility study was carried out to conform that there is no interaction between Glipizide and excipients like HPMC, EC, PVP and lactose because distinct peaks that appear for Glipizide are also found with that of formulation F_8 . Accelerated stability study has been carried out with optimized formulation (F_8) and it was conformed that there is very little change to the physical properties as well as in-vitro release of optimized matrix tablet.

Conclusion

From the results and discussion among the 10 formulations designated as F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9 and F_{10}; the formulation F_8 was found to be successful in terms of sustained release and maximum percentage of drug release with HPMC and EC ratio of 3**:**2. The release profile of that formulation is similar with marketed formulations and can fulfill patient requirements. That formulation is also stable for longer period of time so can be a better formulation for marketing point of view.

Acknowledgement

Author thanks to Sunshine laboratories limited (Hyderabad, India) to provide Glipizide BP pure drug as a gift sample. Thanks to SD fine chemicals, Mumbai for providing HPMC K 15M, EC, PVP and lactose. Author is also thankful to management and principal Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad for providing laboratories and reagents for research work. Thanks to library staff Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad for providing the facility and their active support to carry out literature survey.

References

[1] Gwen MJ., Joseph RR., Banker GS and Rhodes CT, (editors). Modern pharmaceutics. 3rd ed., Morcel Dekker. Inc, 1996, 575.

[2] Chein YW., Novel drug delivery system. 2nd ed. Morcel Dekker. Inc, 1997, 1-42.

[3] Richel WA., Biopharamaceutics and pharmacokinetics aspects in the design of controlled release pre-oral drug delivery system. Drug Dev. Ind. Pham. 15, 1989, 1073-1074.

[4] Lakshmana Murthy G. et. al., Drug release and swelling kinetic studies of Glipizide sustain release matrix tablet-wet granulation method, Int.J.Pharm and Ind. Res., vol-01, issue-01, Jan-Mar 2011.

[5] P.Khemariya et.al, Preparation and in-vitro evaluation of sustained release matrix tablet of Dilitazem. Int. J. of Adv.in.Ph.Sc., 1(2010) 267-273.

[6] Essentials of medical pharmacology, 5th ed., KD Tripathi, Jaypee Brothers, Medical Publication (P) LTD., New Delhi, 246-250.

[7] www. Medicines.org.uk/emc/browsedocuments.

[8] Goodman and Gillman's, The Pharmacological Basis of Therapeutics, 11th ed. Laurence L. Brunton, John S. Lazo, 1635-1638.

[9] T. Ramaji Reddy et.al, Independent release behavior of Glipizide matrix release tablets containing chitosan and xanthum gum, Int.J. on Pharma. And Biomedicals Res. Vol 1(2), 2010, 64-70.

[10] Lachman L, Liberman HA, Kanig JL, The Theory and Practice of Ind. Pharmacy, Mumbai, India; Varghesree Publishers House, 1987: 430-456.

[11] Cooper J., Gunn C., Powder Flow and Compaction. In: Carter SJ, eds., Tutorial Pharmacy. New Delhi, India. CBS Publishers and Distributors: 1986: 211-223.

[12] Martin A., Micromeritics, In: Martin A, eds. Physical Pharmacy. Baltimore, MD: Lippincott Williams and Wilkins: 2001: 423-454.

[13] Aulton ME. Wells TI., Pharmaceutics: The Science of Dosage Form Design, London, England: Churchill Livingstone: 1988: 185-189.

[14] Hadjioannu TP et.al, Quantitative Calculations In Pharmaceutical Practice and Research, New York: VCH Publishers Inc., 1993, 345-348.

[15] Bourne DW, Pharmacokinetics, In: Banker GS and Rhodes CT, (editors). Modern pharmaceutics. 3rd ed., Morcel Dekker. Inc-4, 2002, 67-92.

[16] The Pharmacopeia of India; New Delhi; Controller of Publication, 1996, 736.

[17] Khan K A, Rhodes C T, Evaluation of Different Viscosity Grades of Sodium Carboxy Methyl Cellulose as a Tablet Disintegrants. Phrma Acta. Helv., 1975: 50: 99-102.

[18] Rhodes C T, Carstensen T, Drug Stability Principle and Practice 3rd Ed., New York: 107, 2001, 21-46.

ISSN 0973 – 9874

Isolation and Characterization of Acacetin and β-steroids for the Traditionally used Medicinal Plant *Holostemma ada-kodien*

PATNALA SANTHOSH KUMAR[1*], NARENDRA REDDY G[2], JAYAVEERA K N[3], AND YOGANANDA REDDY K[4]

1. Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapur-515002.
2. Lecturer in Chemistry, Govt. Polytechnic for Women, Suryapet- 508213, Nalgonda(Dist) A.P.
3. Professor& Head, Jawaharlal Nehru Technological University Anantapur, Anantapur-515002.
4. International Science-Tech Research Institute, Vidyuth Nagar, Anantapur-515001.

ABSTRACT

The present study was performed to isolate and characterize the active principles from the traditionally used medicinal plant *Holostemma ada-kodien fam. Asclepiadadeae* commonly known as jivanti. The plant material was subjected for soxhlet extraction using ethanol and pet. ether as asolvent. The preliminary phytochemical studies on the ethanolic and pet ether extract showed the presence of sterol, flavonoids, saponins, steroids, phenols, and terpenoids. The pet ether and ethanolic extract was subjected for column chromatography using silica gel (60-200 mesh size) using gradient elution from non-polar to polar solvent. The fraction yielded two compounds 1 and 2. The isolated compounds were analyzed using physical, chemical and spectral methods and identified as acacetin and β- Steroids.

Introduction

Holostemma ada-kodien is traditionally used medicinal plant belonging to fam. *Asclepiadadeae*. Ancient Ayurvedic scriptures, jivanti is referred to as rasayana (remunerative) and one of the anti-aging herbs. Jivanti has numerous synonyms portraying its peculiarities. There is a mention of another variety of jivanti, called as svarnajivanti, a yellow flowered herb. Maharsi Caraka has categorized jivanti as jivaniya-vitalizing herb. Vayahsthapana – anti aging, svasahara – anti asthmatic and snehopaga – oleating, adjunctive. Susruta has mentioned it as caksusya – beneficial for eyes and tridosaghni – alleviates all the three dosas. Vagbhata has praised it as one of the best amongst vegetables. Bhava prakasa has mentioned it as rasayani-remunerative, balya – a tonic and grahi-anti diarrheal.

Holostemma Creeper is a handsome, extensive, laticiferous, twining shrub with large conspicuous flowers. The bark is deeply cracked. The leaves are ovate to heart-shaped, 5-12 × 2-8 cm, coriaceous, acute, smooth above, and finely pubescent. The flowers are greenish-yellow in color, purplish crimson inside, in lateral cymes. The petals are thick, typical of the milkweed family. Flowers are very fragrant. The central crown is edible. The fruits follicles sub-woody, 6-9 cm long, tapering and green. The roots are pretty long up to a meter or more in length, thick, cylindrical and irregularly twisted. It grows over hedges and in open forests especially on the lower slopes of hills. But its occurrence has diminished very much within this range of distribution and hence it is considered endangered, flowering: april-september. Jivanti is sweet in taste, sweet in the post digestive effect and has cold potency. It alleviates all the three doshas, namely, vata, pitta and kapha. It possesses light and oily attributes. It is a rejuvenative, heart (caksusya and hrdya). It is used in diseases like fever, tuberculosis, burning sensation of the body and raktapittal .

Mainly the roots and the whole plant are used for medicinal purposes. Externally the paste of its leaves and roots alleviate oedema due to vitiation of pitta dosa. The herb is beneficial for external use in various skin diseases, wounds and inflammation of the skin. Internally, jivanti is used in vast range of diseases. The fresh leaves mashed with milk work well as a general tonic and the powder of dried leaves given along with milk ameliorates the burning sensation of the body. In raktapitta, a bleeding disorder, the powder of its leaves, cumin seeds powder and rock candy are given together. Jivanti as a mucolytic, is the best panacea for relieving the phlegm in cough. Because of its astringent property, it works well in colitis. In dysuria, burning micturition and strangury, it eases the problems as

*Address for correspondence

it is diuretic. It nourishes all seven dhatus, especially the fruits, hence bestows aphrodisiac activity. As it is the best refrigerant, it is valuable in fever, dehydration, burning sensation and tuberculosis. Jivanti is one of the best galactogogue herbs, so, benevolent for lactating mothers, in dimness of vision, it improves the eyesight.

Phytosterols and phytostanols, also referred to as plant sterols and stanols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol, but differ from cholesterol in the structure of the side chain. Phytosterols are a natural plant-based ingredient that has been clinically proven to lower cholesterol, thereby reducing the risk of heart disease. Also known as plant sterols, phytosterols work by helping block the absorption of cholesterol in the digestive tract.

A natural product, phytosterols offer a safe and effective way to help reduce the risk of coronary heart disease, the leading cause of death. Phystosterols are found naturally in vegetables and vegetable oils, seeds, nuts and some fruits. They are sometimes incorporated into food products such as margarine, spreads, mayonnaise, yogurt and low-fat milk. Clinical evidence has shown that phytosterols are effective in lowering Low-Density Lipoprotein (LDL) cholesterol. Often referred to as "bad" cholesterol, LDL cholesterol can clog arteries and increase the risk of heart disease.

Acacetin or 5, 7-dihydroxy-4'-methoxy-flavone was classified as a widespread flavones . Several bioactivites of acacetin have been reported by earlier studies. As an antifungal agent, acacetin had a comparable potency with the antifungal. It was reported to be a promising agent for the treatment of atrial fibrillation. As a chemopreventive agent, acacetin had antiproliferative. It inhibited TPA-induced MMP-2 and u-PA expressions of human lung cancer cells.

Experimental

Collection, Identification and preparation of plant materials

The plant were collected from local area of Anantapur, The plant was taxonomically identified, authenticate by HOD, Botany Department, SK university Anantapur. The parts of the plant were manually separated was air dried, powdered, sieved, weighed and stored in air tight container and subsequently referred to as powdered drug.

Extraction and Isolation

Chromatographic separation

Extraction, Isolation and characterization of β- Steroids:

Powdered (2 k) plant parts of *Holostemma ada-kodien* was defatted exhaustively with petroleum ether (60 80^{o}C) in a soxhlet extractor. The solvent was recovered under pressure to obtained dark greenish brown oily mass (5.6g), which was labeled as petroleum ether extracts (PEE) and kept in the refrigerator. The petroleum ether extract of aerial parts of the plant was saponified using 1M alcoholic KOH, to remove fatty material and then subsequently picked up in petroleum ether and the solvent was evaporated to yield 3g of unsaponified matter. This fraction contains lesser number of components than the unsaponified extract.

A small quantity of unsaponifiable matter was dissolved in chloroform and this solution is spotted on TLC plates using precoated aluminium with silica gel 60 F254. Then the TLC plates were run by specific solvent system and viewed individually under UV light and also (5%) sulphuric acid in methanol reagent. Through several pilot experiments it was found that the compounds of unsaponifiable fraction were separated by the solvent system of ethyl acetate and chloroform and ethanol in the proportion of (0.5: 6.4: 3.1 v/v/v). Column chromatography of PEE was conducted using silica gel (Mesh 60 120) that was packed using wet packing method in hexane. The column was run using a mixture of hexane, ethyl acetate by gradient elution technique. TLC was used to monitor the eluates. Similar fractions were pooled together. Further purification is carried out using preparative TLC. Spots were identified, scraped and eluates using petroleum ether and chloroform as solvents.

Finally eluate ST yielded a single spot when subjected to TLC using several solvent systems including ethyl acetate and chloroform and ethanol in the proportion of (0.5: 6.4: 3.1 v/v/v), it showed to be homogenous compound. ST a white crystalline powder **compound 1** (100mg) with melting point (138-140^{o}C) was further subjected to IR, Proton NMR (400MHz), Carbon 13 NMR (100 MHz) and MASS to ascertain the chemical structure [1-4].

Extraction , Isolation and characterization of Acacetin:

Air-dried leaves, weighing 1 k, were soaked in absolute ethanol for 3 days with occasional string. The crude ethanolic extract was filtered, concentrated with a rotary evaporator at temperatures below 60°C, suspended in water, and solvent-partitioned sequentially using Hexane, dichloro methane and Ethyl acetate. Isolation of the flavones was done by column chromatography, with silica gel (100 to 200 mesh), gradient elution was done Hexane, dichloro and Ethyl acetate, mixtures; starting with Hexane – Dichloro methane (9:1). Thin layer chromatography (TLC) was done to monitor the fractionation. The developed chromatograms were visualized with 5% sulfuric acid in ethanol, with which the isolated flavonoids were yellow . Chromatographic fractions that showed yellow spots on their TLC plates were pooled together evaporated dry and resuspended in diethyl ether to yield an yellow color crystals (compound **2**). The purified isolate was also collected, dried in a vaccuum dessicator used for IR, Proton NMR (400MHz), NMR (100 MHz) and MASS to ascertain the chemical structure [5-8].

Results and Discussion

The extract of *Holostemma ada-kodien*, was subjected for various phytochemical tests and the petroleum ether and ethanolic extracts showed the presence of flavonoids, saponins and steroids. The selected extracts were subjected for column separation and the isolated compound was subjected for IR, Proton NMR (400MHz), Carbon 13 NMR (100 MHz) and MASS to ascertain the chemical structure.

IR spectra of isolated compound 1. IR (KBR), OH: 3426, 1056 cm^{-1}, Hydrogen Skeleton: 2935, 2852, 1706, 1462 cm^{-1}, -CH=C=:965, 802 cm^{-1} C=C stretching however this band is weak, at 1457.3 cm 1 is a bending frequency for cyclic (CH2)n and 1381.6 cm 1 for –CH2(CH3)2 γ. The absorption frequency at 1038 cm 1 signifies cycloalkane. The out of plane C H vibration of unsaturated part was observed at 881cm 1.

NMR spectra of isolated compound 1 : ^{1}H NMR (DMSO-d_6).δ 0.8-2.3: (46 H), δ 3.5: (m, 2H, =CH- C$\underline{H}_2$-), δ 5.6: (d, 1H, -CH=C=)

^{13}C NMR: 13C NMR has shown recognizable signals 145.2 and 121.7 ppm, which are assigned C5 and C6 double bonds respectively as in Δ5 Beta steroids 11. The value at 19.32 ppm corresponds to angular carbon atom (C19). Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at δ145.2, 139.8, 121.7 and 118.89

Mass spectrum of isolated compound 1: Mass spectrum (EI. MS): m/z 414 (M+), m/z 396 (M-18), 383 (M-33), 273 (M-side chain), 255 (M-side chain-18), 231 (M-side chain-42), 213 (231-H_2O). All the recorded data such as IR, ^{1}HNMR, 13 C NMR and mass spectrum conclusively prove that the isolated steroidal compound may be β-SITOSTEROL, which was confirmed by standard reference compound and compound data base.

IR spectra of isolated compound -2: IR (KBR), -OH 3450 $cm^{-1,}$ -C=O: 1645 cm^{-1} , -C=C=:1610, 1505 cm^{-1}

NMR spectra of isolated compound -2:

^{1}H NMR (DMSO-d_6).δ 3-3.2: (S, 2H, -OH), δ 3.8: (S, 3H, -OCH_3),δ 5.7: (S, 1H, -CH=C=)'δ 6.3 : (d, 1H, -H-6), δ 6.8: (d, 1H, -H-8)δ 6.9: (d, 1H, -H-6'), δ 7: (d, 1H, -H-5')

C 13 NMR: d 181.717, 164.470, 163.242, 162.282, 161.430, 157.352, 128.290, 122.845, 114.567, 103.647, 103.516, 98.942, 94.048, 55.538.

Mass spectrum of isolated compound -2: Mass spectrum (EI. MS): m/z 285.13 (M+). All the recorded data such as IR, ^{1}HNMR, ^{13}C NMR and mass spectrum were conformed with the reference standards, hence the obtained data are conclusively prove that the isolated steroidal compound may be beta–steroid (compound 1) and Acacetin (compound 2).

Conclusion

The presence of β- steroids and Acacetin, which are antimicrobial, anti-inflammatory and chemopreventive, in the leaves supports the use of *Holostemma ada-kodien* as a medicinal plant. β- steroids, Acacetin and the unidentified compounds in the other fractions could be investigated for significant bioactivities, particularly in relation to the plant's ethnomedicinal use. Moreover, an efficient and healthy method of extracting and purifying β- steroids or acacetin from the leaves of *Holostemma ada-kodien* is developed.

References

[1]. Habib MR., Nikkon F., Rahman ME., and Karim MR. Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantean* (Linn). *Pak. J. Biol. Sci*. 2007; 10: 4174 4176.

[2]. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., chapman and Hall: London. ISBN: 0 412 57270 2, 1998: 302.

[3]. Okunade AL. Ph.D Thesis, University of Ibadan. Nigeria. 1981; 84.

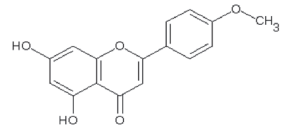
[4]. Abbiw DK. Useful plants of Ghana, Intermediate Tech. Publication. London: Royal Botanic Gardens, Kew. 1990: 207.

[5]. Valant VKM, Wollenweber E (2006). Flavones and Flavonols. In: Andersen M. and K.R. Markham (Eds.) Flavonoids: Chemistry, Biochemistry and Applications. London:Taylor and Francis Group, pp. 628, 644.

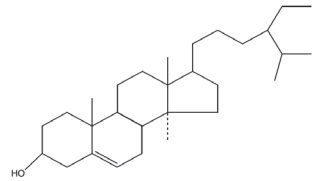
[6]. Agullo G, Gamet PL, Manenti S, Viala C, Remesy C, Chap H, Payrastre B (1997). Relationship between flavonoid structures and inhibition of phosphatidylinositol 3-kinase: A comparison with tyrosine kinase and protein kinase C inhibition. Biochem. Pharmacol., 53: 1649-1657.

[7]. Hsu YL, Kuo PL, Lin CC (2004a). Acacetin inhibits the proliferation of HepG2 by blocking cell cycle progression and inducing apoptosis. Biochem. Pharmacol., 67(5): 823-829.

[8]. Hsu YL, Kuo PL, Liu CF (2004b). Acacetin-induced cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells. Cancer Lett., 212(1): 53-60.



Acacetin



β-steroids

Science – Tech Foundation

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)
(Indexing in CAS)

Plot No. 22, Vidyut Nagar, Anantapur – 515 001(A.P), INDIA

MEMBERSHIP FORM

Dear Editor,

I/We wish to be Annual Member / Life Member of Journal of Pharmacy and Chemistry (An International Research Journal of Pharmaceutical and Chemical Sciences) and agree to abide by your rules and regulations

1. Name in full: _____ Male ☐ Female ☐
   (In block letters)

2. Nationality: _____ 3. Date of birth:_____

3. Corresponding Address: _____

   _____

   _____

5. Institutional / Business Address: _____

6. Phone : (O): _____ (R): _____ E-mail :_____

7. Academic Qualifications : _____

8. Profession and Experience : _____

9. Present Position / Post : _____

10. Pharmaceutical / Chemical interest: _____

Membership Fees	**Life**	**Annual**
Individual	Rs. 8000/- US $ 800	Rs. 1500/- US $ 150
Institutional	Rs. 20,000/- US $ 2000	Rs. 2000/- US $ 200

Rs. / $ _____ remitted by Bank / Cash

DD No. _____ Date _____ Banker's Name_____

DD's are drawn in favour of **Science-Tech Foundation**, Payable at **Anantapur.**

Place: _____

Date: _____

(Signature of the applicant)

Note: (i) All authors should be member of this Journal, (ii) Saperate Photostat copy may be filled and send.

INSTRUCTION TO AUTHORS

GENERAL REQUIREMENTS: Journal of Pharmacy and Chemistry (ISSN 0973-9874) is a quarterly Journal, ***Indexing in CAS(Coden:JPCOCM)*** which publishes original research work that contributes significantly to further the scientific knowledge in Pharmaceutical Sciences (Pharmaceutical Technology, Pharmaceutics, Biopharmaceutics, Pharmacokinetics, Pharmaceutical Chemistry, Computational Chemistry and Molecular Drug Design, Pharmacognosy and Phytochemistry, Pharmacology, Pharmaceutical Analysis, Pharmacy Practice, Clinical and Hospital Pharmacy, Cell Biology, Genomics and Proteomics, Pharmacogenomics, Stem Cell Research, Vaccines & Cera, Bioinformatics and Biotechnology of Pharmaceutical Interest) and in Chemical Sciences (Inorganic, Soil, Forensic, Analytical, Nano, Environmental, Polymer, Physical, Agricultural, Medicinal, Biochemistry, Organic, Computational, Food, Pesticides etc). Manuscripts are accepted for consideration by Journal of Pharmacy and Chemistry on the condition that they represent original material, have not been published previously, are not being considered for publication elsewhere, and have been approved by each author. Review articles, research papers, short communication and letters to the editor may be submitted for publication.

SUBMISSION OF MANUSCRIPTS: Typewritten manuscripts prepared using MS Word should be submitted in triplicate and RW-CD to Prof. Dr. K.N Jayaveera, Editor-in-Chief of Journal of Pharmacy and Chemistry , Plot No 22, Vidyut Nagar, Ananthapur- 515 001, Andhra Pradesh, India. e-mail: jpcanantapur@gmail.com

All components of the manuscript must appear within a single electronic file: references, figure legends and tables must appear in the body of the manuscript.

TYPING INSTRUCTION: The following detailed instructions are necessary to allow direct reproduction of the manuscript for rapid publishing. If instructions are not followed, the manuscript will be returned for retyping. The following typefaces, in 12 points size, are preferred: Times Roman.

GENERAL FORMAT: The typing area must be exactly 6 5/8" (168 mm) wide by 9 7/8" (250 mm) long. Justify margins left and right (block format). The entire typing area of each page must be filled, leaving no wasted space. Text should be double-spaced, special care should be taken to insure that symbols, superscripts and subscripts are legible and do not overlap onto lines above or below. Make sure text lines are equidistant.

TITLE: On the first page of the manuscript, start title 1" (25 mm) down from top text margin. Type title in all capital letters, centred on the width of the typing area and single-spaced if more than one line is required. The title should be brief, descriptive and have all words spelled out. Double-space, then type the author(s) name(s), single-spaced if more than one line is required. Double-space, than type author(s) address(es), single-spaced, capitalizing first letters of main words. Quadruple-space before Abstract.

ABSTRACT: Centre, type and underline abstract heading, capitalizing the first letter. A double-space should separate the heading from the abstract text. Indent abstract text approximately 1/2" (13 mm) from both left and right margins. The abstract should be intelligible to the reader without reference to the body of the paper and be suitable for reproduction by abstracting services. Introduction to the text (without a heading) should being four spaces below the abstract using full margins.

KEY WORDS: Three or more key words must be provided by authors for indexing of their article. Key words will be listed directly below the Abstract. Abbreviated forms of chemical compounds are not acceptable. Spell out entirely, using the official nomenclature. Example: L-dihydroxyphenylalanine (L-DOPA)

MAJOR HEADINGS: Papers must include the major headings: Introduction, Methods, Results, Discussion, Acknowledgments and References. Capitalize first letter, underline, and centre headings on width of typing area.

TABLES/FIGURES: Incorporate tables and/or figures (B & W) with their legends into the main body of text.

REFERENCES: Referneces should be referred to a number [1] in the text and be listed according to this numbering at the end of the paper. Only papers and books that have been published or in press may be cited; unpublished manuscripts or manuscripts submitted to a journal but which have not been accepted may not be cited.

The references shoulld comprise the following information and in the given order and with given punctuation as given in the example below: Author name (s), Initials (s), Publication Title, Page Number, Year of Publication.

Standard Journal Article:

[1] Bhattacharyya D, Pandit S, Mukherjee R, Das N, Sur TK. Indian J Physiol Pharmacol 2003; 47:435.

[2] Skottova N, Krecman V. Physiol Res 1998; 47:1.

Book:

[1] Ghosh MN. Fundamentals of Experimental Pharmacology, 2nd ed. Calcutta Scientific Book Agency, 1984:154.

Proofs will be sent to the corresponding author. These should be returned as quickly as possible.

The facts and view in the article will be of the authors and they will be totally responsible for authenticity, validity and originality etc. the authors should give an undertaking while submitting the paper that the manuscripts submitted to journal have not been published and have not been simultaneously submitted or published elsewhere and manuscripts are their original work.

www.stfindia.com

This Quarterly Journal is Printed, Published, Owned and Edited by Dr. K.N. Jayaveera, Published from Science-Tech Foundation, Plot No.22, Door No. 12/3/925, Vidyut Nagar, Anantapur - 515 001. Send your queries at www.stfindia.com and Printed at Vipla Computer Services (Designers & Multi Colour Offset Printers) # 1-8-725A/1/A, Balaji Bhagyanagar Apartments, Nallakunta, Hyderabad - 500 044. Ph. 040-27676910, 27677078.