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MESSAGE FROM SECRETARY

It is a matter of great happiness to me to know that student and faculty of Geethanjali are bringing out an excellent piece of scientific and technical magazine “PHARMAGAZINE”. As I understand , this demonstrates the literary, art, imaginative, technical and scientific skills of our students and faculty.

My heartfelt congratulations to the entire team for keeping the tradition high and raising the bar of the presenting the articles. Keeping this in mind. I expect the contributions to this magazine to be of very high standard and quality.

I Wish all the success for this venture.

G.R.RAVINDER REDDY
M.Tech (NIT), Ex IPS
Secretary
Geethanjali College o Pharmacy

MESSAGE FROM PRINCIPAL

Spell bound by the efforts that our students and faculty have shown, I take immense pleasure appreciating them for coming up with the first edition of the technical and scientific magazine. Their enthusiasm has enraptured me and took me back to my Pharmacy days.

No child is a congenital Einstein or Newton. In fact, it is through one's consistent hard work and passion to do something that takes one to glorious heights.

Hope the scientific and technical Magazine being published stands elite among the other magazines published in some of the colleges apparently to spread knowledge and help students to get acquainted with the state of the art of science and technology. It even helps in developing one's leadership skills and team spirit. I congratulate all of them for their untiring efforts and would advise the students to utilize this opportunity. My best wishes to the editorial members of the scientific and technical Magazine "PHARMAGAZINE" and hope to see it flourishing , inculcating creativity and innovation in the pursuit of spreading knowledge.

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DEPARTMENT OF BIOTECHNOLOGY

PHARMACY PRACTICE: A BOON TO THE SOCIETY

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Today, Pharmacy practice plays an important role in any country as they take responsibility for patient's medicine related needs for access to healthcare. The role of the pharmacist has expanded significantly from the traditional concept of medication dispensing. Today, pharmacists provide more direct patient care through services rendered directly to the patient at the pharmacy. However, in India only the supply of medicines remains the core activity of the community pharmacist. The role of the pharmacists in the community, and with it their medicine management, may change in the wake of the rapid growth of domestic medicine output and national healthcare expenditure. In any health system, it is essential that patients have access to reliable information and competent professional care. After physicians and nurses, pharmacists constitute the third largest group of health professionals.

Pharmacy Practice in India

India is a developing nation that is home to over 1.3 billion people. Rapidly growing, the country accounts for 2.4% of the world's surface but is home to 17.71% of the world's population. Throughout its 28 states, 9 union territories and 22 national languages. The genesis of community pharmacy practice in India can be traced back to British India when allopathic drugs were introduced and were made available through drug stores towards the end of the nineteenth century. The practice of prescribing and dispensing was normally a function performed by doctors. In addition, most doctors trained their clinic assistants to dispense medicines and assist in the compounding of medicinal preparations, who were popularly known as "compounders". After the enforcement of provisions of the Pharmacy Act 1948, pharmacists working in India must have a pharmacist registration certificate issued by the state in which they wish to practice. To obtain a registration certificate, the prospective pharmacist must acquire the minimum diploma from a pharmacy institute that is recognized by the Pharmacy Council of India.

During the early period the diploma courses were mostly run by Government medical colleges. Since the 1980's there has been phenomenal growth of private institutions offering D. Pharm. courses. However, most of these self- financing institutions that provide education in pharmacy are away from practice environment resulting in diploma pharmacists lacking the skills needed for the community practice setting.

Pharmacy Practice in USA

Point of Care Testing (POCT)

The Clinical Laboratory Improvement Amendment (CLIA) of 1988 was created more than 30 years ago. However, there has been a recently renewed interest in CLIA-waived POCT in pharmacy due to advances in the testing technology and an increased need for accessible testing. The nation is currently facing a shortage of primary care doctors, and as a result, pharmacists are being viewed as viable candidates to offer health screenings for potentially serious but not yet diagnosed conditions. The National Community Pharmacist Association states that CLIA-waived POCT includes, but is not limited to, testing for acute and chronic conditions such as influenza, group A strep, HIV, hepatitis C, dyslipidemia, and diabetes. While these common tests are extremely valuable, pharmacists must remain responsive to the changing needs of patients as new situations and challenges develop in 2018.

According to the CDC, there has been a 400% increase in acute hepatitis C virus infection among people aged 18 to 29 and a 32.5% increase among those aged 30 to 39, which highlights the need for accessible testing. This is especially true given the communicable nature of hepatitis C and the fact that about half of adults with HCV do not know they have it.

Specialty Pharmacy

Specialty pharmacies focus primarily on providing medication and drug therapy management for patients with specific disease states, or who require drugs needing special handling or with a high price. Disease states such as cancer, HIV/AIDS, hepatitis, immune disorders, infertility, and others are managed by a team of healthcare providers to ensure optimum treatment for each patient. A report from IMS Institute for Healthcare Informatics projects that, in 2020, 28% of global spending will be for specialty medications, up from 26% in 2015. Pharmacists have prepared for specialty roles primarily through residency or certificate training programs. In the 2012 APhA Career Pathway Evaluation Program survey, 30% of 27 specialty pharmacist respondents indicated they had completed a residency and 30% said they completed a certificate training program before becoming a specialty pharmacist.

Current advances in pharmacy training and continued growth in the prevalence of rare diseases will undoubtedly result in an increase in the number of specialty pharmacies providing high-touch high-cost medications. As these numbers increase, there will be a larger need for pharmacists who are fully trained in this growing area of pharmacy practice.

Provider Status

Several key pharmacy organizations are working together to achieve provider status for pharmacists through many routes. Pharmacists are providing high level patient care such as medication therapy management, disease state education, health screenings, and wellness services such as immunizations. According to APhA, attaining provider status will increase patient access to specialty pharmacies, which will create an incentive for pharmacists to provide even more high-level care services.

Competency-based education

The mission of pharmacy education is to prepare graduates who provide patient-centered care that ensures optimal medication therapy outcomes and provides a foundation for specialization in specific areas of pharmacy practice; to participate in the education of

patients, other healthcare providers; to conduct research; and to provide service and leadership to the community.

The Educational Outcomes are the competencies that should be achieved by a graduate of a professional degree program in pharmacy prior to entering the work force. A pharmacist is able to provide Patient Care in cooperation with patients, prescribers, and other healthcare practitioners by utilizing sound therapeutic principles and evidence based data, while always emphasizing legal, ethical, social, economic, and professional issues surrounding technology and evolving clinical sciences that may impact therapeutic outcomes. Also, upon graduation, a pharmacist is expected to be competent in Systems Management which includes managing resources in cooperation with patients, prescribers, and other healthcare providers, as well as administrative staff to promote health and provide, assess, and coordinate medication distribution. Finally, a pharmacist must also be able to promote Public Health by improving health, wellness, and disease prevention.

Pharmacy practice analyses, comprises a detailed competency statement in three main areas:

- Assure Safe and Effective Pharmacotherapy and Optimize Therapeutic Outcomes
- Assure Safe and Accurate Preparation and Dispensing of Medications
- Provide Healthcare Information and Promote Public Health

Clinical Pharmacy

Clinical pharmacy is a health science discipline in which pharmacists provide patient care that optimizes medication therapy and promotes health, wellness, and disease prevention. As a discipline, clinical pharmacy also has an obligation to contribute to the generation of new knowledge that advances health and quality of life. They possess in-depth knowledge of medications that is integrated with a foundational understanding of the biomedical, pharmaceutical, socio-behavioral, and clinical sciences. To achieve desired therapeutic goals, the clinical pharmacist applies evidence-based therapeutic guidelines, evolving sciences, emerging technologies, and relevant legal, ethical, social, cultural, economic, and professional principles.

In accordance, clinical pharmacists assume responsibility and accountability for managing medication therapy in direct patient care settings, whether practicing independently or in consultation or collaboration with other health care professionals. They routinely provide medication therapy evaluations and recommendations to patients and health care professionals. Clinical pharmacists are a primary source of scientifically valid information and advice regarding the safe, appropriate, and cost-effective use of medications.

Clinical pharmacist

Clinical pharmacists work directly with physicians, other health professionals, and patients to ensure that the medications prescribed for patients contribute to the best possible health outcomes. Clinical pharmacists are educated and trained in many direct patient care environments, including medical centers, clinics, and a variety of other health care settings.

Duties of Clinical Pharmacist

Clinical pharmacists:

- Assess the status of the patient's health problems and determine whether the prescribed medications are optimally meeting the patient's needs and goals of care.
- Evaluate the appropriateness and effectiveness of the patient's medications.

- Recognize untreated health problems that could be improved or resolved with appropriate medication therapy.
- Consult with the physicians and other health care providers in selecting the medication therapy.
- Advise the patient on how to best take their medications.
- Support the health care team's efforts to educate the patient on other important steps to improve or maintain health, such as exercise, diet, and preventive steps like immunization.

How do clinical pharmacists care for patients?

Clinical pharmacists:

- Provide a consistent process of patient care that ensures the effectiveness, and safety of the patient's medication use.
- Consult with the patient's physicians and other health care providers to develop and implement a medication plan.
- Apply specialized knowledge of the scientific and clinical use of medications; including medication action, dosing, adverse effects, and drug interactions, in performing their patient care activities in collaboration with other members of the health care team.
- Apply their clinical experience to solve health problems through the rational use of medications.

Where do you find a clinical pharmacist?

Clinical pharmacists practice in many health care environments, including hospitals and their affiliated outpatient clinics, emergency departments, community pharmacies, physicians' offices, community-based clinics, nursing homes, and managed care organizations.

Pharmacists in Academia, Health Organization Management, Regulatory Oversight, Pharmacy Organizations, and Industry

Many pharmacists pursue a career that allows them to help facilitate the attainment of the goals of the profession through academic, organizational, and government initiatives that are focused on advancing the full scope of pharmacy practice. Pharmacists who choose to combine practice with academia have a responsibility to develop practice, research, and teaching role models in evolving healthcare settings. As university faculty members, pharmacists have multiple paths from which to choose, faculty positions vary greatly. But, in general, they include an integration of practice activities with clinical programs, research, teaching, mentoring, consulting, scholarship, community and campus-based service, and collaboration with other health science programs.

Preparation for academic positions may include additional work through postgraduate education or training programs, either academic degree programs (e.g., Masters or PhD) or professional programs (i.e., residencies, fellowships). Pharmacists also hold positions within government agencies and nongovernmental organizations, such as the US Public Health Service, Commissioned Service (Food and Drug Administration, Indian Health Service and Bureau of Prisons), the National Institutes of Health, the Armed Services, State boards of pharmacy, and the World Health Organization. They serve in a multitude of capacities

relying on their pharmacy degree as a foundation for the particular area in which they are working.

In a nutshell, India faces massive challenges in providing health care for its vast and growing population. Despite many barriers, community pharmacy services are central to the safe and effective medicines management in advancing health. With rapidly occurring changes in the health care delivery and growing patient expectations, it is hoped that community pharmacy practice will change accordingly.

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

CHEMISTRY INVENTIONS TODAY- DEVICE TO REMOVE CARBON DIOXIDE FROM AIR

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The technique is based on passing air through a stack of charged electrochemical plates. The device is essentially a large, specialized battery that absorbs carbon dioxide from the air (or other gas stream) passing over its electrodes as it is being charged up, and then releases the gas as it is being discharged.

Artificial photosynthesis

Scientists have doubled the efficiency of a chemical combo that captures light and splits water molecules so the building blocks can be used to produce hydrogen fuel. Their approach provides a platform for developing revolutionary improvements in so-called artificial photosynthesis -- a lab-based mimic of the natural process aimed at generating clean energy from sunlight.

Chiral compounds

Photochemical deracemization of chiral compounds achieved. Enantiomeric molecules resemble each other like right and left hands. Both variants normally arise in chemical reactions. But frequently only one of the two forms is effectual in biology and medicine

C-H functionalization

Chemists at The Scripps Research Institute (TSRI) have devised a new and widely applicable technique for building potential drug molecules and other organic compounds. Their newest tool improves a basic molecule-building operation called C-H functionalization

Artificial leaf

A widely-used gas that is currently produced from fossil fuels can instead be made by an 'artificial leaf' that uses only sunlight, carbon dioxide and water, and which could eventually be used to develop a sustainable liquid fuel alternative to petrol.

Nano confined reactions

Georgia State University chemistry researchers have unlocked one of the mysteries of catalytic reactions on a microscopic scale, allowing for the design of more efficient industrial processes. The researchers established a new imaging strategy that can track single molecules as they shimmy through tiny pores in the shells of silica spheres and monitor the chemical reaction dynamics on catalytic centers at the core, producing the first quantitative measurements of how confinement on a nano scale actually speeds up catalytic reactions.

IN-VITRO CYTOTOXIC ACTIVITY EVALUATION OF SOME N-SUBSTITUTED 2-THIOXOTHIAZOLIDIN-4-ONE DERIVATIVES

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Abstract
A series of new N-substituted 5-arylidenerhodanine derivatives (D1-D10) were synthesized starting from 5-arylidenerhodanines. The 5-arylidinerhodanines were reacted with 2- bromo-1-phenylethanone in presence of potassium hydroxide and dimethyl formamide under microwave conditions to give N-substituted 5-arylidenerhodanine derivatives. The synthesized compounds were confirmed on the basis of spectral data and elemental analyses. The synthesized compounds evaluated for their cytotoxic activity against two different human cancer cell lines using MTT assay. The cytotoxic activity results revealed that the synthesized compounds demonstrated the ability to inhibit HT-29 (colon cancer), MCF-7 (breast cancer) cancer cell lines against reference drug Methotrexate. Among the compounds tested, the most active compounds (4-trifluoro, 2-thienyl and 4-fluoro-substituted) possess significant cytotoxic activity against both the cancer cell lines and all other compounds showed moderate to weak activity.

Key Words: N-substituted rhodanine, Cytotoxic activity, HT-29 (colon cancer) and MCF-7 (breast cancer) cancer cell lines

Introduction

Rhodanine (2-thioxothiazolidin-4-one) is considered as privileged scaffold in drug discovery and receiving considerable attention in medicinal chemist community due to their broad therapeutic activities such as antimicrobial,antiviral, antidiabetic and anticancer activity. Additionally, rhodanine-based molecules have been popular as small molecule inhibitors of numerous targets such as HCV NS5B protease, HCV NS3 protease, aldose reductase,] β-lactamase, UDP-N-acetylmuramase/L-alanine ligase, fungal protein mannosyl transferase-1 (PMT1), cathepsin-D, anthrax lethal factor protease, histidine decarboxylase, JNK-stimulating phosphatase-1 (JSP-1),and phosphodiesterase (PDE-4). Among the thiazolidine derivatives, numerous compounds containing thiazolidine-2,4-dione and rhodanine have been recognized as new potential anticancer agents. For example, GSK1059615 (Fig 1) is a potent, reversible, ATP-competitive, thiazolidinedione inhibitor of PI3K α. In our previous work, we found that 5-arylidenerhodanine analogues exhibited considerable cytotoxicity against 3-different cancer cell lines and taking account of the revealed cytotoxic activity, we assumed that the N-substituted 5-arylidenerhodanine derivatives would enhance activity. We report in this paper, the details of synthesis of N-substituted 5-arylidene rhodanine derivatives and their cytotoxic activities.

Experimental

All the chemicals and solvents were analytical grade and used without further purification. Melting points were determined on open capillaries, using Boitus melting point apparatus, expressed in ºC and are uncorrected. 1H NMR spectra of the compounds were recorded on Bruker AMX-400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. Infrared spectra were recorded in KBr disc on Bruker ALPHA-T FTIR spectrophotometer. The mass spectrum of the compounds were recorded either on Agilent-1100 ESI-Mass (Turbo Spray) Spectro photometer. Microanalyses were carried out with a Perkin-Elmer model-2400 series II apparatus and were within ± 0.4% of the theoretical values. Column chromatography was performed on silica gel (230-400 mesh). General procedure for the synthesis of 5-arylidenerhodanines (C1- C10) by Knoevenagel

condensation: A mixture of rhodanine 1 (20 mmol), respective aromatic aldehyde 2 (20 mmol) and NH2SO3NH4 (ammonium sulphamate) was subjected to microwave irradiation (MWI) at 600 watts intermittently at 30 sec intervals for specific time (3-6 min) (Fig.2). Completion of the reaction was identified by TLC using silica gel-G. After completion of the reaction, the mixture was poured onto crushed ice, and the solid that separated was isolated by filtration, dried and recrystallized from ethanol to give desired product (C1- C10). The physical and spectral characterization data represented in Table 1 and 2. General procedure for the reaction of 5-arylidenerhodanine with 2-bromo-1- phenylethanone. To a solution of 5-arylidenerhodanine (1mmol) in DMF (5 mL) was added fine dispersed anhydrous potassium hydroxide (2 mmol). The mixture was stirred for 30 minutes at room temperature to give the potassium salt of 5-arylidenerhodanine. To the resulting suspension as added 2-bromo-1-phenylethanone (1 mmol) and then the reaction mixture was irradiated in microwave at 800W for 4-10 min. Thin layer chromatography (TLC) was employed to monitor the progress of reaction. After completion of the reaction, the reaction mass was poured into icecold water and stirred continuously. The resulted compound was washed with water and ethanol, dried and then recrystallized from absolute ethanol. The physical and spectral characterization data represented in Table 2 and 3. Biological activity Cell lines and cell cultures HT-29 (colon cancer) and MCF-7 (breast cancer) cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MEM (Minimum Essential Media Eagle), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis, MO). Fetal bovine serum (FBS) was purchased from Arrow Labs, 96 well flat bottom tissue culture plates were purchased from Tarson. Cytotoxicity assay The in vitro cytotoxicity of each synthesized compounds (D1 to D10) was evaluated by using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based colorimetric assay [22] and reported in Table 4. The cells were seeded in 96 well plates at a density of 1x104 (counted by Tryphan blue exclusion dye method) per well and were incubated in the 5% CO2 incubator for 24 h to enable them to adhere properly to the 96-well polystyrene micro plate. After incubation the medium was replaced with fresh media containing different dilutions of the test compounds. Then the plates were incubated for additional 48 hrs at 370C in DMEM/MEM with 10 % FBS medium. Following incubation, the medium was removed and replaced with 90 µl of fresh DMEM without FBS. To the above wells, 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) was added and incubated at 370C for 3-4 hrs, there after the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals) and incubated at 370C for 10 min. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a SpectraMax 190 Microplate Elisa reader (Molecular Devices Inc. USA) at 570 nm. Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC50 (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumor cells by 50 % as compared to the control untreated cells. IC50 values were determined from the plot: % inhibition versus concentration. The inhibitory concentration (IC) values were calculated as follows: % inhibition = [1-OD (570 nm) of sample well/OD (570 nm) of control well] X 100 RESULTS AND DISCUSSION i) Chemistry The synthesis of N-substituted-5-arylidenerhodanines was accomplished by reacting 5- arylidenerhodanine with 2-bromo-1-phenylethanone in presence of potassium hydroxide in DMF in microwave condition. This reaction strategy was very facile (4-10 min) and provided very good yields (64-78%). The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (1H-NMR) of all the synthesized compounds were in full agreement with proposed structures. The 1H NMR spectra of the N-substituted-5-arylidenerhodanines revealed the characteristic methylenic proton in between δ 7.6 and 7.9. The spectra also showed the peaks accounting for the aromatic protons and for the different substituent's

present in between the corresponding regions of the spectrum. ii) Cytotoxic activity the synthesized compounds D1 to D10 have been evaluated for in vitro cytotoxic activity against two human cancer cell lines HT-29 (colon cancer) and MCF-7 (breast cancer) using MTT assay as reported in Table 4. The results clearly revealed that most of the compounds possessed cytotoxic activity as evidenced by t he IC50 values. Among all the compounds tested against HT-29 and MCF-7 cell lines among them were D7 , D6 and D9 exhibiting moderate to good antitumor activity against HT-29 (IC50 = 19 µg/mL, 23 µg/mL and 25 µg/mL respectively), MCF-7 (IC50 = 26 µg/mL, 30 µg/mL and 33 µg/mL respectively) and the result which is compared with methotrexate. The other compounds also showed activity but at a higher IC50 values. The results clearly indicated the importance of fluorine substitution on 5-aryl ring in enhancing the cytotoxic activity. CONCLUSION In the present study we have demonstrated a simple, efficient and cleaner strategy for the synthesis of N-substituted-5-arylidenerhodanines by reacting 5-arylidenerhodanines with 2-bromo1-phenylethanone in presence of potassium hydroxide in DMF by employing microwave irradiation. Moreover, the base used is easily available, inexpensive, eco-friendly, facile work-up, which makes the reaction convenient, more economic, and environmentally benign. With encouraging cytotoxic activity results, all the synthesized compounds need to be evaluated in terms of active concentration and also examine the mechanism of compounds responsible for mediated cell proliferation inhibition, cell cycle distribution using flow cytometry. All the synthesized compounds can be further explored for structural modifications and studies concerning the structure-activity relationships are in progress in our laboratory.

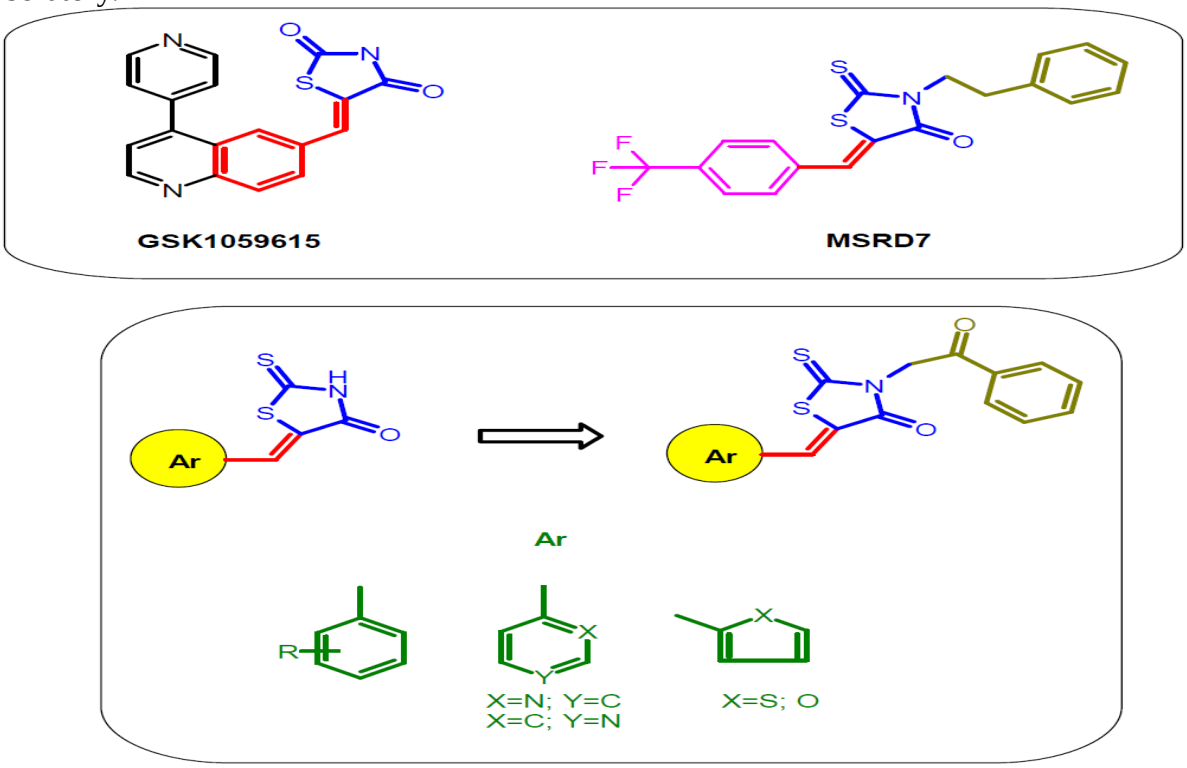

Fig 1. Structure of some previously reported cytotoxic agents and background for target compounds synthesis.

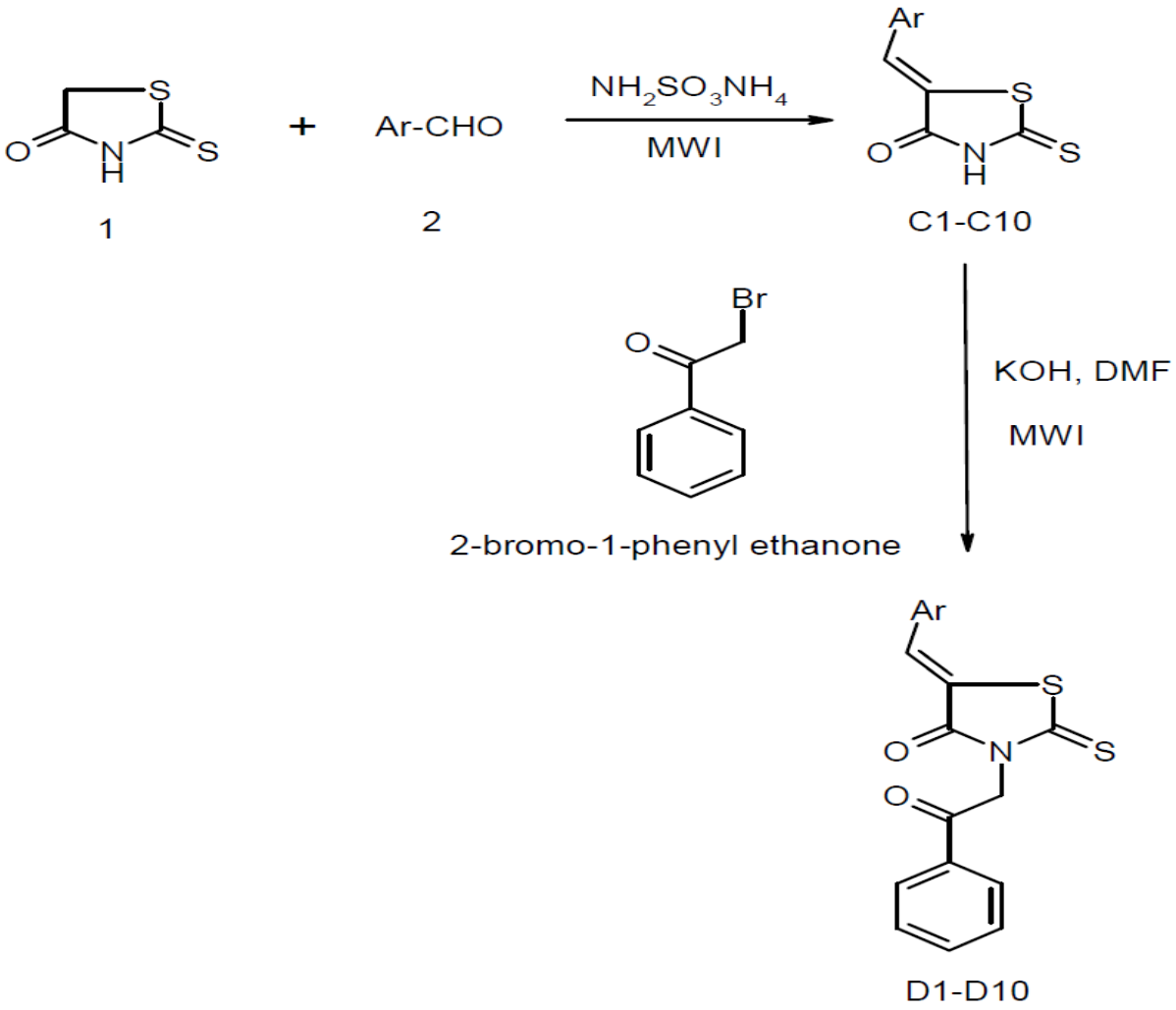

Fig 2. General scheme for synthesis of N-substituted 5-arylidenerhodanine

Table 1. Physical characterization data of 5-arylidenerhodanines (C1-C10)

Compound	Ar	Molecular Formula	Molecular weight	Melting point (°C)	Yield %
C1	Phenyl	$C_{10}H_7NOS_2$	221	208°C	68%
C2	2-Chlorophenyl	$C_{10}H_6ClNOS_2$	254.99	232°C	67%
C3	4-Pyridyl	$C_9H_6N_2OS_2$	221.99	292°C	58%
C4	3-Methoxyphenyl	$C_{11}H_9NO_2S_2$	251.01	232°C	78%
C5	2-Furyl	$C_8H_5NO_2S_2$	210.98	246°C	65%
C6	2-Thienyl	$C_8H_5NOS_3$	226.95	253°C	56%
C7	4-Trifluorophenyl	$C_{11}H_6NOS_2F_3$	288.98	246°C	63%
C8	2-Pyridyl	$C_9H_6N_2OS_2$	221.99	264°C	57%
C9	4-Fluorophenyl	$C_{10}H_6NOS_2F$	238.99	196°C	68%
C10	2-Naphthyl	$C_{14}H_9NOS_2$	271.01	246°C	72%

Table 2. Physical characterization data of N-substituted-5-arylidenerhodanines (D1-D10)

Compound	Ar	Molecular Formula	Molecular weight	Melting point (°C)	Yield %
D1	Phenyl	$C_{18}H_{13}NO_2S_2$	339.44	162	75%
D2	2-Chlorophenyl	$C_{18}H_{12}ClNO_2S_2$	373.88	212	65%
D3	4-Pyridyl	$C_{17}H_{12}N_2O_2S_2$	340.43	231	67%
D4	3-Methoxyphenyl	$C_{19}H_{15}NO_3S_2$	369.46	167	74%
D5	2-Furyl	$C_{16}H_{11}NO_3S_2$	329.40	178	64%
D6	2-Thienyl	$C_{16}H_{11}NO_2S_3$	345.46	139	68%
D7	4-Trifluorophenyl	$C_{19}H_{12}NO_2S_2F_3$	407.44	126	78%
D8	2-Pyridyl	$C_{17}H_{12}N_2O_2S_2$	340.43	181	71%
D9	4-Fluorophenyl	$C_{18}H_{12}NO_2S_2F$	357.43	219	69%
D10	2-Naphthyl	$C_{22}H_{15}NO_2S_2$	389.50	242	73%

Table 3: Spectral data of some synthesized compounds

Compound	IR in KBr cm^{-1}	^{1}H-NMR ($CDCl_3$ / TMS) δ ppm
C1	1234 (N-C=S), 1697 (N-C=O), 1586 (N-H), 3054 (Ar-CH)	7.65(1H, s, =CH), 7.50-7.67 (5H, Ar-H), 13.85 (1H, s, N-H).
C2	1233 (N-C=S), 1696 (N-C=O), 1589 (N-H), 3072 (Ar-CH), 849 (C-Cl)	7.67 (1H, s, =CH), 7.53-7.77 (4H, Ar-H), 13.96 (1H, s, N-H).
C3	1242 (N-C=S), 1700 (N-C=O), 1596 (C=N), 3014 (Ar-CH)	7.58 (1H, s, =CH), 7.54-8.74 (4H, Ar-H).
C4	1213 (N-C=S), 1685 (N-C=O), 1584 (N-H), 3154 (Ar-CH), 1166 (O-CH_3)	7.4 (1H, s, =CH), 7.08-7.63 (4H, m, Ar-H), 3.82 (3H, s, OCH_3), 13.83 (1H, s, N-H)
C6	1186 (N-C=S), 1680 (N-C=O), 1578 (N-H), 3056 (Ar-CH), 665 (C-S)	7.94 (1H, s, =CH), 7.32-8.10 (3H, Heteroaryl-H), 13.80 (1H, s, N-H).
C8	1233 (N-C=S), 1715 (N-C=O), 1596 (C=N), 3113 (Ar-CH)	7.67 (1H, s, =CH), 7.42-8.8 (4H, Heteroaryl-H), 13.6 (1H, s, N-H).
D4	1221 (N-C=S), 1688 (N-C=O), 3155 (Ar-CH), 1162 (O-CH_3)	7.60 (1H, s) 7.34-7.18 (6H, m), 7.03-7.01 (1H,d), 6.92 (2H,s) 4.72 (2H, s), 3.79 (3H, s).
D5	1191 (N-C=S), 1687 (N-C=O), 3058 (Ar-CH)	7.63(1H, s), 7.38 (1H, s), 7.3-7.1 (5H, m), 6.76 (1H, m), 6.52 (1H, m), 4.27-4.6 (2H, s).
D8	1235 (N-C=S), 1720 (N-C=O), 1577 (C=N), 3122 (Ar-CH)	8.69 (1H, s), 8.57-8.56 (1H, d), 7.70-7.68 (1H, d), 7.59 (1H,s), 7.36-7.33 (1H, dd), 7.26-7.16 (5H, m), 4.72 (2H, s).
D9	1236 (N-C=S), 1696 (N-C=O), 3024 (Ar-CH), 1122 (C-F)	7.56-7.49 (3H,m), 7.36-7.13 (7H, m), 4.7 (2H, s).
D10	1235 (N-C=S), 1688 (N-C=O), 3046 (Ar-CH)	7.90-7.79 (5H, m), 7.53-7.46 (3H, m), 7.29-7.199 (5H, m), 4.7 (2H, s).

Table 3: Spectral data of some synthesized compounds

Compound	IR in KBr cm^{-1}	^{1}H-NMR ($CDCl_3$ / TMS) δ ppm
C1	1234 (N-C=S), 1697 (N-C=O), 1586 (N-H), 3054 (Ar-CH)	7.65(1H, s, =CH), 7.50-7.67 (5H, Ar-H), 13.85 (1H, s, N-H).
C2	1233 (N-C=S), 1696 (N-C=O), 1589 (N-H), 3072 (Ar-CH), 849 (C-Cl)	7.67 (1H, s, =CH), 7.53-7.77 (4H, Ar-H), 13.96 (1H, s, N-H).
C3	1242 (N-C=S), 1700 (N-C=O), 1596 (C=N), 3014 (Ar-CH)	7.58 (1H, s, =CH), 7.54-8.74 (4H, Ar-H).
C4	1213 (N-C=S), 1685 (N-C=O), 1584 (N-H), 3154 (Ar-CH), 1166 (O-CH_3)	7.4 (1H, s, =CH), 7.08-7.63 (4H, m, Ar-H), 3.82 (3H, s, OCH_3), 13.83 (1H, s, N-H)
C6	1186 (N-C=S), 1680 (N-C=O), 1578 (N-H), 3056 (Ar-CH), 665 (C-S)	7.94 (1H, s, =CH), 7.32-8.10 (3H, Heteroaryl-H), 13.80 (1H, s, N-H).
C8	1233 (N-C=S), 1715 (N-C=O), 1596 (C=N), 3113 (Ar-CH)	7.67 (1H, s, =CH), 7.42-8.8 (4H, Heteroaryl-H), 13.6 (1H, s, N-H).
D4	1221 (N-C=S), 1688 (N-C=O), 3155 (Ar-CH), 1162 (O-CH_3)	7.60 (1H, s) 7.34-7.18 (6H, m), 7.03-7.01 (1H,d), 6.92 (2H,s) 4.72 (2H, s), 3.79 (3H, s).
D5	1191 (N-C=S), 1687 (N-C=O), 3058 (Ar-CH)	7.63(1H, s), 7.38 (1H, s), 7.3-7.1 (5H, m), 6.76 (1H, m), 6.52 (1H, m), 4.27-4.6 (2H, s).
D8	1235 (N-C=S), 1720 (N-C=O), 1577 (C=N), 3122 (Ar-CH)	8.69 (1H, s), 8.57-8.56 (1H, d), 7.70-7.68 (1H, d), 7.59 (1H,s), 7.36-7.33 (1H, dd), 7.26-7.16 (5H, m), 4.72 (2H, s).
D9	1236 (N-C=S), 1696 (N-C=O), 3024 (Ar-CH), 1122 (C-F)	7.56-7.49 (3H,m), 7.36-7.13 (7H, m), 4.7 (2H, s).
D10	1235 (N-C=S), 1688 (N-C=O), 3046 (Ar-CH)	7.90-7.79 (5H, m), 7.53-7.46 (3H, m), 7.29-7.199 (5H, m), 4.7 (2H, s).

The Novel Biomarkers in Diabetes

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Abstract

According to International Diabetes Federation, the worldwide prevalence of impaired glucose tolerance (IGT) in adults is 318 million and is expected to reach 482 million by 2040. With increasing burden of prediabetes and their expectant progression in diabetes has compounded the problem. Now question is that how we can identify the subjects at high risk to develop prediabetic state and among them who will rapidly progress into diabetes? Once a person diagnosed to be a diabetic then there are only few marker which can depict development of diabetes related complications and also to help in preventing such diabetes related complication progression. In this article, we will review several biomarkers used to predict the risk of progression to prediabetes, diabetes states in context to their mechanism of action, sensitivity, specificity, advantages, disadvantages and association with dysglycemia. The risk stratification arising due to insulin resistance by novel biomarker will improve clinical outcome both in prediabetics and diabetics.

Introduction

In coming days diabetes Mellitus will be a major health problem for the world, with its highest impact on newly industrialized, developing nations and minority groups in developed countries.[1] Diabetes will increase from 135 to 300 million worldwide between 1995 and 2025, of which (93 - 97%) will be type II diabetic patients mounting a 42% increase in diabetes and overall 27% increase in the prevalence globally. Not only diabetics but the pre diabetics will be compounding the problem. According to Centers for Disease Control one out of three, adults had prediabetes which is an intermediate state and agonizingly, 90% were unaware of their diagnosis. In 2019, the International Diabetes Federation estimated that the worldwide prevalence of impaired glucose tolerance (IGT) in adults was 318 million and expected to reach 482 million by 2040. The subject in question is how can we identify patients with prediabetes early and can we prevent progression to diabetes? Identification of these prediabetes states and risk stratification arising due to insulin resistance by novel biomarker will improve clinical outcome both in diabetics and pre diabetics. Diabetes Prevention Program has shown that changes in dietary habits, weight loss, and increased

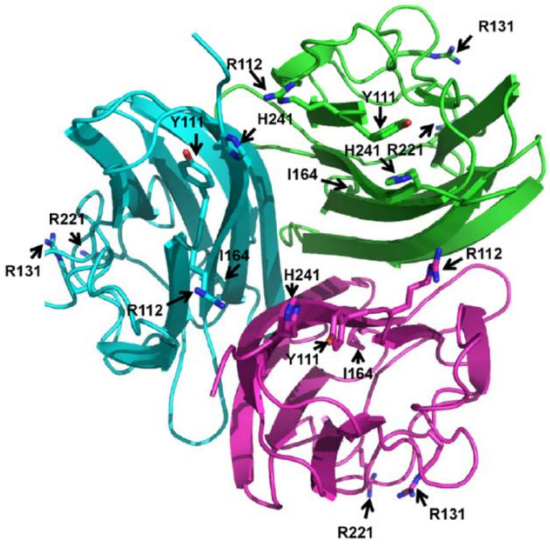
physical activity reduced the risk of progression to diabetes. So, the tools to identify and making an individual aware of his prediabetes state is need of time.

Biomarkers for risk stratification diagnose prediabetes and prevent complication in diabetes. Factors leading to prediabetic state are genetics, peripheral IR, defects in insulin secretion, glucotoxicity, lipotoxicity, impaired incretin release, amylin accumulation, inflammation, oxidative stress, and decreased β-cell mass leading to β-cell dysfunction. Prediabetes includes isolated impaired fasting glucose (IFG) or impaired glucos tolerance (IGT). However the differing criteria of WHO and ADA made issue controversial as slight changes in criteria leads of large long term outcome. Hence review of biomarker will give better understanding of disease course and therapeutic interventions.

Now the Novel Biomarkers

Adiponectin

A helper from fat tissue Adiponectin, is formed from adipose tissue, it has insulin sensitizing, anti-inflammatory, and anti-atherogenic functions and it is shown to be independent predictor of diabetes. Concentrations of adiponectin are inversely related to IR (insulin resistance) and obesity. Lower level of adiponectin was observed even a decade prior to development of diabetes or its complications especially in men. In offspring of diabetic parents, the baseline adiponectin levels are inversely related to the risk of prediabetes and it is independent of sex or ethnicity. Hyperinsulinemic euglycemic clamp and intravenous glucose tolerance test showed that adiponectin levels were directly correlated with higher insulin sensitivity and indirectly with insulin concentration.



Three dimensional structures of modeled human adiponectin globular domain and positions of amino acid variations. Human adiponectin was constructed by homology modeling using mouse adiponectin as a template. Each monomer of the adiponectin trimer structure is shown in different color. The positions of amino acids being substituted are indicated with arrows.

Fetuin-A

Fetuin-A (FetA) is a glycoprotein secreted from liver,it correlates with increased risk of T2DM incidence and it's complications. Importantly, un like adiponectin, the EPICPotsdam prospective cohort study establish, FetA as a independent risk marker after normalization of the BMI and waist circumference for T2DM. FetA promotes lipid-induced IR through the toll-like receptor 4 (TLR 4) - inflammatory signaling pathway leading to production of inflammatory cytokines. High-fat diet-fed FetA knock down animal module have less TLR4-mediated signaling in adipose tissue causing IR, with FetA injection in this model induces inflammatory signaling and IR. Presence of FetA and TLR4 both needed for FFA (free fatty acid) induced inflammatory cytokine expression in adipocytes. Higher FetA is also correlating with risk of cardio vascular disease in candidates susceptible IR. In conclusion FetA acts as an endogenous ligand for TLR4 for induction of IR by lipids. Hence FetA may therefore serve as a novel therapeutic target for IR.

Metabolites and amino acid: The hidden catalyzer

Amino acids: Branched chain amino acids (BCAAs): The good one and the bad one for diabetes Isoleucine, leucine, valine, tyrosine, aromatic amino acid phenylalanine and glycine have been significantly associated with development of diabetes. Glutamine, methionine, cysteine, and 2-aminoadipic acid are increased in initial insulin-resistant states. Contrarily glycine levels are lower in prediabetic individuals. These changes in circulating amino acid levels may prove to be significant predictive biomarker for IR and T2DM.

α-Hydroxybutyrate (α-HB)

α-Hydroxybutyrate (α-HB) is a catabolic by product of threonine , methionine and glutathione anabolism (cysteine formation) in hepatic tissue. Increased oxidative stress and lipid oxidation leads to chronic shifts in glutathione synthesis resulting in elevated α-HB levels in individuals of IR. It is reflected by increased urinary α-HB excretion in IR. α-HB can be used as a biomarker to distinguish NGTinsulin- sensitive (NGT-IS) individuals from IGT and IFG individuals and NGT-IS individuals from those with NGT-IR individuals.63 Hence it can be an effective and promising biomarker for prediabetes.

Lipoprotein(a)

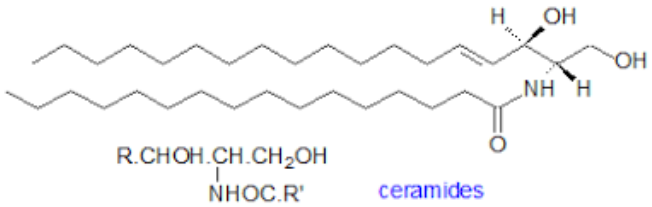
Lipoprotein(a) is synthesized by liver. Elevated levels of LP(a) is proved to be independent risk factor for development of CVD. Serum Lp(a) and the prevalence of prediabetes and T2DM have inverse relationship. Although the mechanism is not clear higher insulin may play a role in reducing Lp(a) concentration.

Triglycerides and high-density lipoprotein

In prediabetics significant increment in levels of small HDL3 particles compared to HDL-C levels have been observed. Small HDL3 particles is positively relates with triglyceride and negatively relates with HDL-C. HDL-C induces insulin secretion and low HDL-C promotes progression of prediabetes to diabetes however, it is not clear whether HDL-C levels plays a role in β-cell dysfunction or not.

Ceramide

Cerami dealipid molecules mediate I R . It acts through inhibiting insulin action by decreasing phosphorylation. further it accumulates in insulin-resistant tissues and induce inflammation through activation of TNF-α. Studies also showed, ceramide propagates coronary artery disease.



Ferritin and transferring

Storage and iron release are regulated through an intracellular protein ferritin. There is a association of high serum ferritin and transferrin saturation with increased risk of prediabetes and diabetes. Mechanism being the catalytic iron induces formation of reactive oxidative molecules causing hepaticdys function, and β-cell apoptosis, which contribute to IR. Dietary iron restriction prevents the development of diabetes and loss of β-cell function. However the threshold levels of ferritin which correlate with IR is not certain.

Mannose binding lectin serine peptidase and thrombospondin-1

High levels of MASP1 found in prediabetes, diabetes, and the CVD. Even onset of prediabetes and IR occurred earlier in those with higher MASP1 plasma levels. Elevated FPG and 2-hour glucose levels have positive association with higher levels of MASP1. Other markers like thrombospondin1(THBS1) and glycosylphosphatidylinositolspecific phospholipase D1 (GPLD1) are also increased in prediabetes. Thrombospondin have inflammatory properties, and contributes to higher prediabetes prevalence.

Acyl-carnitine

Serum levels of acyl-carnitines have been shown to be elevated in prediabetes. Although the role of acylcarnitine in FAO and its mechanism in IR are not clear. It has been postulated that abnormal of FAO and mitochondrial function leads to accumulation of intermediary products such as acyl-carnitines which promotes inflammation and IR.

MicroRNAs: The hidden player

MicroRNAs (miRNAs) are small, noncoding RNAs participating in post-transcriptional gene expression. These are involved in many biological processes such as growth, development, differentiation, proliferation, and cell death. Recently, miRNAs have been studied in pre-diabetes and found to be strongly correlated. In particular miR-192 and miR-193b high levels observed in prediabetics. miR-193b plays critical roles in differentiation of brown adipocytes and inflammation reduction in IR. Elevated levels of both miRNAs i.e., miR-192 and miR-193b were observed with IFG and IGT and it also correlated with Tg levels and the fatty liver index in animal models. It is quite significant as a fatty liver can be associated with prediabetes.86 Other miRNAs significantly elevated in T2DM are miR-9, miR-29a, miR-30d, miR-124a, miR-146a, and miR-375, all of these play a role in β-cell dysfunction. These miRNAs negatively regulate insulin expression and secretion. Few miRNAs levels are low in prediabetes, of these microRNA-126, miRNA-15a is found in endothelial cells and it is quite low in IGT/IFG and T2DM. miR-15a is thought to regulate and promote insulin formation by inhibiting endogenous uncoupling protein-2 gene expression and increasing insulin secretion.88 So, miR-15a have a significant role in β-cell function and insulin synthesis..

Inflammatory markers: The universal culprits

IL-6 and CRP higher concentration is associated with a greater risk of diabetes development. These inflammatory markers are useful in identifying individuals at higher risk of developing T2DM. Tissue plasminogen activator-1 (PAI-1) changes is an independent predictor of incidence of diabetes. IL-18 level increased parallel to progression from prediabetes to diabetes in the Gutenberg study. Levels of IL-1RA were found to be significantly elevated even 13 years prior to the diagnosis of diabetes and it raises more rapidly about 6 years prior to diagnosis even after adjusting for obesity. The Whitehall Study, showed an increase in IL-1RA in prediabetes in parallel with decreasing insulin sensitivity, increasing β-cell function, and 2-hour glucose levels, all of which occurred altogether years before the development of T2DM. **White blood cell count, fibrinogen, and hematological indices**

Subtle indicator A high WBC countpredicts worsening insulin action, insulin secretion, and diabetes development in Pima Indians. The neutrophillymphocyte ratio (NLR) has also been associated with both microvascular and macrovascular complications in diabetes.

Conclusions and Prospective

Dysglycemia is a continuous pathophysiologic process. It is overtly underestimated and puts large number of individuals at risk for full blown disease state. With development of hyperglycemia it is already late in the evolution to T2DM leading to uninhabitable micromacrovascular complications. β-cell function markedly reduced leading to progressively rising glucose levels, on higher side of "normal glycemic range". So there is a vital need to identify and use sensitive precise biomarkers to predict progression to dysglycemia at the earliest, when β-cell function is optimally functional. Interference at this stage may be more responsive to lifestyle modification and pharmacological agents. A well identified set of biomarkers in a clinical practice will give better sensitivity and specificity in prediabetes and diabetes complication prediction.

Comparative studies of biomarkers will help to ascertain their clinical utility. Furthermore, genetic studies assessing mutations will also provide additional insight into associations with metabolic deregulation.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF CHALCONES AND PYRAZOLINES

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Abstract:

Chalcones are 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α,β- unsaturated carbonyl system. They have displayed a broad spectrum of biological activities. In this view it was proposed to synthesize some novel pyrazolines from chalcones. Chalcones are prepared by treating 2-acetyl-5-bromothiophene with different aromatic compounds. These chalcones on condensation with phenyl hydrazine HCl. pyridine as a catalyst gave 3- (5-bromothiphene-2yl)-1-phenyl-1H-pyrazole derivatives. The synthesized compounds have been characterized by their melting point, TLC, IR and 1H NMR spectral data. They have been screened for their antibacterial activity against Gram positive bacteria *B.subtillis* & *B.pumilus* and Gram negative bacteria *E. coli* & *P.vulgaris* and antifungal against *A.niger* & *p.crysogenium*.

Keywords: Pyrazolines, Chalcones, 2-acetyl-5-bromothiophene, 3-(5-bromothiphene-2yl)-1-phenyl-1H-pyrazole.

Introduction

Pyrazolines and Chalcones were reported to possess various biological activities. In the present communication we report the synthesis of novel pyrazolines via chalcones [1-6]. Hence, chalcones are important intermediates in the synthesis of various heterocyclic ring compounds like pyrazolines, pyrimidines, isoxazolines and thiazolines etc. Therefore the present research work is viewed on the synthesis of Pyrazolines via chalcones by claisen-schmidt condensation using 3-acetylpyridine with either aromatic or heteroaromatic aldehydes in the presence of alkali [7-15]. The resulting chalcones after purification and characterization by physical and spectral methods have been successfully converted into novel substituted pyrazolines by reaction with phenyl hydrazine hydrochloride in absolute ethanol. The structures of the various synthesized compounds were assigned on the basis of elemental analyses, IR, ^{1}H NMR, and mass spectral data. These compounds were screened for their antimicrobial activity [16].

Materials and methods

Melting points were determined on a capillary melting point apparatus and are uncorrected. 1H NMR and 13C NMR spectra were recorded in the indicated solvent on Bruker AMX 400 MHz spectrophotometer using TMS as an internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer BXF1 spectrophotometer. Microanalyses were performed on carlo Ebra 1108 element analyzer and were within the ± 0.5% of the theoretical values. Column chromatography was performed on silica gel (Merck, 100- 200 mesh)[17-23].

Experimental section

General procedure for the synthesis of pyrazolines from chalcones:

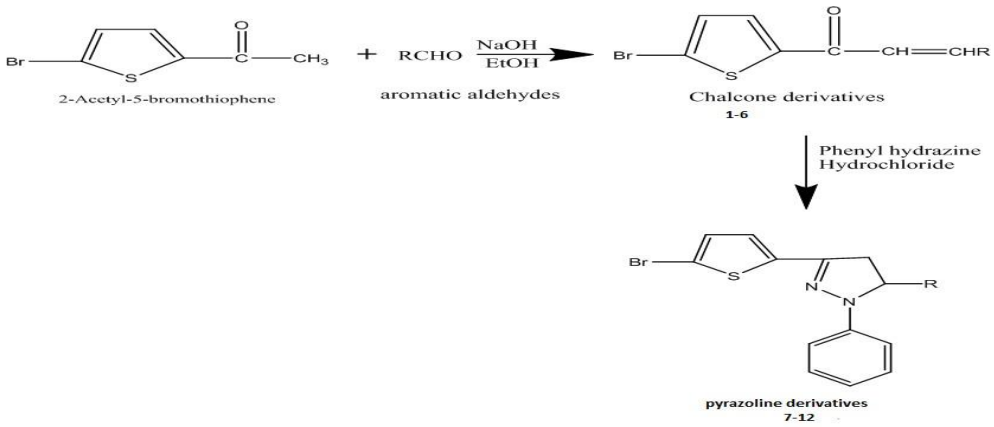
Step-1: Synthesis of Chalcones:

A mixture of 2-acetyl-5-bromothiophene (0.01 Mol) and benzaldehyde derivative (0.01 Mol) was stirred in ethanol (25 mL) and then aqueous solution of 10% potassium hydroxide (6mL) was added to it. The mixture was kept overnight at room temperature. After completion of the reaction, it was poured into crushed ice and acidified with dil. HCl. The chalcone precipitated out as solid. Then it was filtered, dried and purified by column chromatography using hexane and ethylacetate mixture (90:10) as mobile phase [24, 25].

Step-2: Synthesis of Pyrazolines via Chalcones:

To a solution of Chalcone (0.001 Mol) and phenyl hydrazine Hcl (500 mg) in 20 mL ethanol, pyridine (0.3mL)was added as catalyst. The mixture was refluxed for 5hrs and the solvent was evaporated completely using rotary evaporator. The reaction mixture was poured in to ice water and the solid mass separated was filtered, dried and purified by column chromatography with N-hexane/EtOAC and recrystalised from chloroform to give white crystallised needles [26-28].

Reaction



Results and Discussion

Table 1: Antibacterial activity of Chalcones (Compounds 1- 6):

Compound code	Zone of inhibition (in mm)							
	B. subtilis		*B. pumilis*		*E. coli*		*P. vulgaris*	
	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
Standard	28	33	31	32	25	27	28	31
Control	-	-	-	-	-	-	-	-
1	16	16	10	17	15	19	12	17
2	15	22	16	18	15	23	12	19
3	18	18	13	18	16	18	12	18
4	17	18	15	19	18	17	12	20
5	15	18	12	18	16	19	12	21
6	20	20	12	20	18	18	14	21

Note: " No zone of inhibition"

Table 2: Antibacterial activity of Pyrazolines (Compounds 7– 12):

Compound code	Zone of inhibition (in mm)							
	B.subtilis		*B.pumilis*		*E.coli*		*P.vulgaris*	
	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
Standard	28	33	31	32	25	27	28	31
Control	-	-	-	-	-	-	-	-
7	16	18	13	13	14	16	13	17
8	11	19	11	12	10	13	12	18
9	10	20	12	14	11	20	12	18
10	13	20	11	13	12	17	13	17

11 12	12 13	16 20	12 14	14 13	13 12	20 20	12 14	20 21

Note: – " No zone of inhibition"

From the above results it is evident that all the synthesized compounds showed antibacterial and antifungal activities at both 50µg (0.05 ml) and 100µg (0.1 ml) dose levels but less than that of the benzylpencillin and fluconazole used as standards for antibacterial and antifungal activities respectively. Among the compounds tested, 4,5,6,9,12 were found to be more potent antibacterial compounds and 8,12 exhibited the highest and 1,3 shows moderate antifungal activity. However, in particular pyrazoline containing chlro (9) substitution at para position on phenyl ring enhanced both the antibacterial and antifungal activities. The standard drugs used were Benzylpencillin and Fluconazole for antibacterial and antifungal activity respectively.

Summary and conclusion

The title demonstrates "An Efficient Synthesis and ''Synthesis and antimicrobial activity of chalcones and pyrazolines'' and through the synthesis of chalcones by 2- acetyl-5-bromo- thiophene with different aldehydes. The formed chalcones were treated with phenyl hydrazine hydrochloride in presence of pyridine

The proposed compounds were synthesised successfully and characterized by Melting point, recrystallisation, ^{1}HNMR, IR, spectroscopy. All the synthesized compounds were subjected to antibacterial and anti-fungal activity.

The chalcones and pyrazoline derivatives evaluated for antibacterial activity and they were effective against *B.pumilis, B. subtilis,* and *E.coli, and P.vulgaris* at both the concentration levels when compared with penicillin-G as standard references.

It is interesting to note from the result of antifungal evaluation of chalcones and pyrazolines are effective against *A.niger, p.crysogenum* when compared with fluconazole as reference standard. From the above results, it is interesting to note that the challcones and pyrazolines, which are having electron releasing substituent's like chlorine, fluorine, methoxyl at C-4 position of aromatic ring-B showed moderate to considerable antibacterial and antifungal activities, when compared to that of heteroaryl chalcones and pyrazolines.

DEPARTMENT OF PHARMACEUTICS

PHARMACEUTICAL NANOTECHNOLOGY: OVERCOMING DRUG DELIVERY CHALLENGES IN CONTEMPORARY MEDICINE

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Abstract
Nanomedicine is set to bring advantages in the fight against unmet diseases. The field is recognized as a global challenge, and countless worldwide research and business initiatives are in place to obtain a significant market position. However, nanomedicine belongs to those emerging sectors in which business development methods have not been established yet. Open issues include which type of business model best fits these companies and which strategies would lead them to sustained growth. This paper describes the financial and strategic decisions by nanomedicine start-ups to reach the market successfully, obtain a satisfactory market share, and build and maintain a competitive defendable advantage. Walking nanomedicine-product from the hands of the inventor to those of the doctor, we explored the technological transfer process, which connects laboratories or research institutions to the marketplace. The process involves detailed analysis to evaluate the potentials of end-products, and researches to identify market segment, size, structure, and competitors, to ponder a possible market entry and the market share that managers can realistically achieve at different time horizons. Attracting funds is crucial but challenging. However, investors are starting to visualize the potentials of this field, magnetized by the business of "nano."

1. Introduction

Globally defined as the application of nanotechnology to the clinical arena, nanomedicine has its roots in the same basic concepts and principles of nanotechnology; that is, materials with the nanoscale features present unique characteristics, otherwise absent at a macroscopic level. Just as nanotechnology benefits from mathematics and engineering, nanomedicine too has a multidisciplinary nature involving notions and techniques borrowed from biology, chemistry, and physics. As a result of this successful marriage, nanostructure materials display emerging functions that have exceptional benefits when applied to medical devices.

The success of nanotechnology in the healthcare sector is driven by the possibility to work at the same scale of several biological processes, cellular mechanisms, and organic molecules; for this reason, medicine has looked at nanotechnology as the ideal solution for the detection and treatment of many diseases. One of the many applications of nanotechnology to the medical sector is in the field of drug delivery. The advent of protocols and methods for the

synthesis, functionalization, and use of nanoparticles and nano-carriers has flooded the scientific and clinic community with new therapeutic approaches from molecular targeting to radiofrequency ablation and from personalized therapies to minimally invasive techniques.

While most members of the investment community are able to grasp the meaning of nanotechnology and can expertly launch and manage a viable product into the market, they are limited in their conceptual understanding of this scientific discipline and the intricate inner workings behind the product's functionality. On the contrary, those involved in the scientific research recognize that nanomedicine is an expansion of nanotechnology but has very little understanding of the business expertise required to develop their technologies into a commercial product. Cooperation is therefore needed between the two factions in order to lead nanomedicine-based inventions to a successful market position.

2. Challenges in Delivery of Contemporary Therapeutics

Drug discovery process has been in forefront utilizing recent advances in molecular biology, - together with medicinal chemistry, protein structure based screening, and computational analysis, as part of rational approach to discovering drug molecules that will address unmet clinical needs. For example, proteins identified from structural biology platform can serve as targets for discovering new drug molecules. The discovery of antisense oligonucleotides (ASN), plasmid DNA (pDNA), peptides and protein therapeutics has also shown a greater potential in treating several complex diseases. In general, all recent drugs have shown a great potential in the clinical management of several complex diseases like cancer, metabolic diseases, auto-immune diseases, cardiovascular diseases, eye diseases, neurodegenerative disorders and other illness.

2.1 Chemical Challenges

Physico-chemical properties impact on both pharmacokinetics and pharmacodynamics of the drug in vivo, and must be considered when selecting a suitable delivery method. The chemical challenges faced by small and macromolecular drugs (ASN, pDNA, peptides, proteins, siRNA and miRNA) are many folds, which mainly include:

(i) Molecular size

(ii) Charge

(iii) Hydrophobicity

(iv) In vivo stability

(v) Substrate to efflux transporters

2.2 Remote Disease Targets

Anatomical and physiological barriers involved in the body restrict the direct entry of small and macromolecular drugs into the target extracellular or intracellular tissue locations resulting in sub-optimal doses at target site and reduced efficacy. However, cytotoxic drugs and RNA therapeutics have their target sites inside the cells, therefore need to be delivered intracellular in sufficient doses to produce therapeutic effect. The first limiting anatomical

barrier for orally administered drugs is epithelial lining of gut walls, where from drugs will permeate through either by transcellular or paracellular transport. Therefore, altering the chemical properties by making the drugs in salt form, encapsulating in DDS based on cyclodextrins, lipid or polymeric carriers, or using permeability enhancers could promote bioavailability of drugs. Cytochrome P450 and efflux transporters present in the enterocytes of intestinal walls also forms as another limiting barrier to drug permeability. Use of cytochrome P450 and efflux pump inhibitors can promote oral drug absorption. For example, pre-treatment with curcumin results in inhibition of P-gp and cytochrome P450 expression in the GI tract, leading to increased oral bioavailability and efficacy of drugs.

3. Nanotechnology Solutions

The science of nanotechnology has begun just in the last decade, but in this short time, it has been successfully applied in several fields ranging from electronics to engineering to medicine. Recent understanding of cellular barriers and molecular profile of diseases, and controlled manipulations of material at the nanometer length scale, nanotechnology offers great potential in the disease prevention, diagnosis, and treatment. Nanotechnology has also allowed for challenging innovations in drug delivery, which are in the process of transforming the delivery of drugs. Nanosystems fabricated using controlled manipulation of material are exploited for carrying the drug in a controlled manner from the site of administration to the target site in the body. They are colloidal carriers with dimensions <1,000 nm and can traverse through the small capillaries into a targeted organ down to target cell and intracellular compartments, which represent the most challenging barrier in drug targeting.

The critical attributes of any nanoparticle DDS are to

(1) Protect a labile drug molecule from both in vitro and in vivo degradation,

(2) maintain the effective pharmacokinetic and biodistribution pattern,

(3) Promote drug diffusion through the epithelium, and/or

(4) Enhance intracellular distribution.

However, the specificity, sensitivity and simplicity are very important for any nanosystem to be clinically successful as a DDS.

3.1 Enhancing Solubility and Permeability

Solubility and permeability are two of the most critical biopharmaceutical characteristics impacting the successful delivery of drug molecules through anatomical membranes in the body. If the drug molecule is not a substrate to efflux transporters and metabolizing enzymes, then the solubility (hydrophilic and hydrophobic) plays a major role in determining oral intestinal permeability. Biopharmaceutical Classification System (BCS) is proposed based on the solubility and permeability properties of the drugs which classifies drugs into one of four classes.

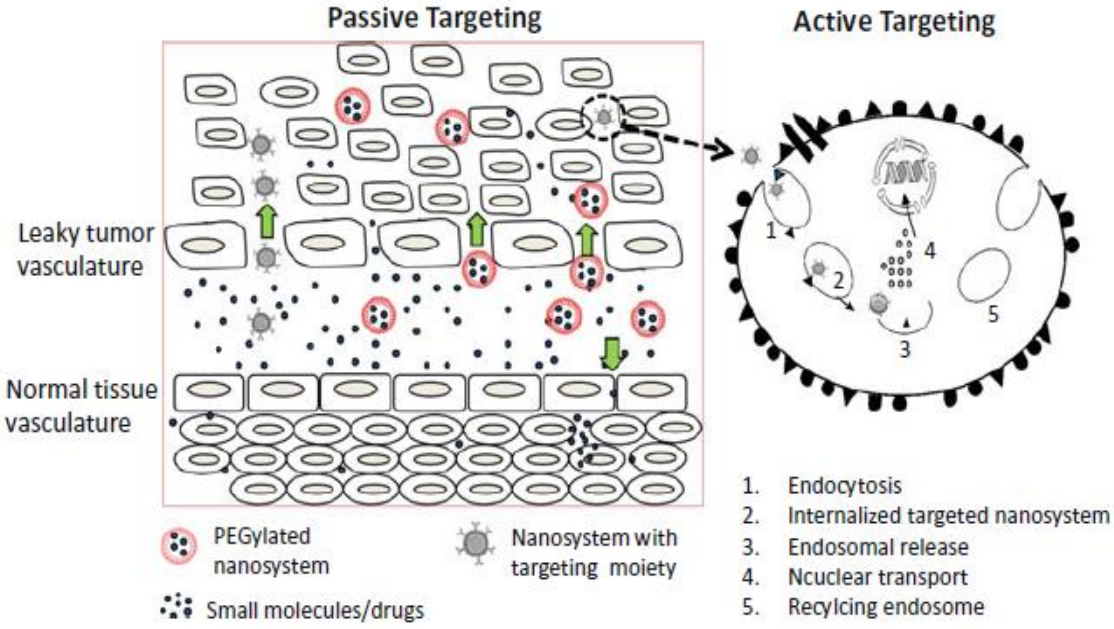
- Class I drugs are highly soluble and permeable in the GI tract, therefore, bioavailability is not an issue with Class I drugs.

- Class II drugs are poorly aqueous soluble but highly lipophilic. They are well permeable across the GI tract due to high lipophilicity, but the bioavailability is likely to dissolution rate limited due to low aqueous solubility.
- Class III drugs are highly soluble but have low permeability due to their low lipophilicity. In both Class II and Class III examples, DDS plays a critical role in overcoming poor solubility and permeability.
- On the other hand, Class IV drugs show low solubility and low permeability, and exhibit poor and variable bioavailability. Methods to enhance both solubility and permeability should be adopted for these drugs.

3.2 Targeted Delivery to Disease Sites

Targeted delivery exploiting the structural changes and cellular markers of a given pathophysiology can potentially reduce the toxicity and increase the efficacy of drugs. This is highly important in case of diseases like cancer, where dose-limiting toxicities and drug resistance constitute major barriers to drug success. General targeting mechanisms consists of passive and active targeting.

Schematic illustration of passive and active targeting strategies in tumor drug delivery



3.3 Intracellular and Subcellular Delivery

The nanosystems once in the disease vicinity, they need to enter the cells and transfer the payload to sub-cellular organelles. There are two mechanisms playing a role in intracellular and subcellular delivery is non-specific or specific uptake of nanosystems by cells.

- In case of non-specific uptake, cells surround the nanosystems and forms a vesicle in the cell called an endosome. The endosomes then fuse with the highly acidic organelles called lysosome, which are rich in degrading enzymes. Endosomes usually travels in a specific direction and join at the nuclear membrane.

- Specific uptake on the other hand, involves receptor mediated endocytosis, where the actively targeted nanosystem binds to the cell-surface receptor, resulting in internalization of the entire nanoparticle-receptor complex and vesicular transport through the endosomes. The receptor can be re-cycled back to the cell surface following dissociation of complex. After the cellular internalization, stability of the payload in the cytosol and delivery to specific organelles, such as mitochondria, nucleus etc, is also essential for therapeutic activity. However, many drugs do not survive in the lysosomal environment.

3.4 Enabling Non-invasive Delivery

Non-invasive delivery is an alternate to systemic delivery of drugs, and mainly includes drug delivery via intranasal, pulmonary, transdermal, buccal/sublingual, oral and trans-ocular routes. Patient compliance has been found to be much higher when drugs given by non-invasive routes and therefore they are considered to be a preferred route of drug delivery. However, the preferred route of administration for a given drug selected based on several factors, such as biopharmaceutical properties (solubility, permeability and stability) of a drug molecule, disease state, onset of action, dose frequency and adverse effects.

4. Illustrative Examples of Nanotechnology Products

Nanotechnology based concepts have been extensively applied in engineering of nanosystems for delivery of contemporary therapeutics in a controlled manner from the site of administration to the target disease in the body. The history of nanosystems reaches back to 1950s when the first polymer-drug conjugate was reported with N-vinyl pyrrolidine conjugated to glycyl-L-leucine-mescaline. However, the most relevant nanosystems were conceptualized only after the first report of liposomal preparations in 1964 and their subsequent use as vehicle for drug delivery application. Soon after, synthesis of albumin nanoparticle was reported in early 1970s with a subsequent early attempt of exploiting them as the first protein based DDS. The pharmacological effects of polymer-based nanoparticles were studied and their application as DDS envisioned around the same time.

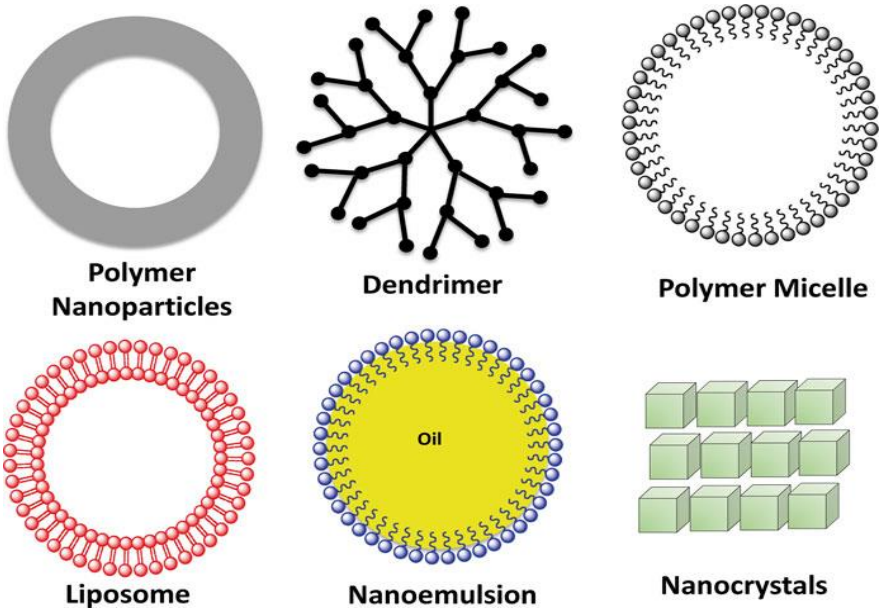
Several different types of nanosystems have been researched and much focus has specifically been on tailoring the size, physical properties and surface functionality of the delivery systems for varying therapeutic applications. The collective research input on the nanotechnology based improvement of DDS has enabled several products in to the market in the past two decades.

Nanotechnology – based products in clinical application

Nanotechnology platform	Trade name	Active agent	Indication(s)
Liposomes	Ablelcet	Amphotericin B	Fungal infection
	AmBisome	Amphotericin B	Fungal infection
	Amphotec	Amphotericin B	Fungal infection

	Daunoxome	Daunorubicin	Antineoplastic
	DepoCyt	Cytarabin	Lymphomatous meningitis
	Doxil/Caelyx	Doxorubicin	Antineo plastic
	Myocet	Doxorubicin	Antineoplastic
	OncoTCS	Vincristine	Non-Hodgkin's lymphoma
Micelles	Estrasorb	Estradiol	Vasomotor symptoms
Nanocrystal	Emend	Aprepitant	Antiemetic
	Tricor	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia
	Triglide	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia
	Megace ES	Magesterol acetate	Anorexia, cachexia or an unexplained significant weight loss in AIDS patients
	Rapamune	Sirolimus	Immunosuppressant
Nanoemulsion	Tocosol	Paclitaxel	Nonsuperficial urothelial cancer
Nanoparticle	Abraxane	Paclitaxel	Metastatic breast cancer
Nanotube	Somatulinedepot	Lanreotide	Acromegaly
Superparamagnetic iron oxide	Feraheme injuction	Ferumoxytol	Treatment of iron deficiency anemia in patients with chronic kidney disease
	Feridex	Ferumoxide	MRI contrast agent
	GastroMARK	Ferumoxsil	Imaging of abdominal structures

Different types of pharmaceutical nanosystems used in drug and gene delivery



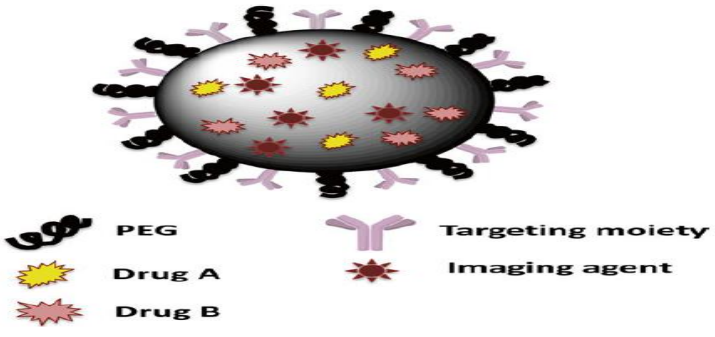
5. Multifunctional Nanotechnology

Biological system presents several barriers to effective drug delivery. It is therefore germane to develop drug delivery strategies to circumvent these barriers. This could be achieved by making the right choice of material as delivery vehicle, surface modification to increase targeting and intracellular availability of the drug and improving the functionality of the delivery system to achieve the diagnostic applications. The nanosystem with these multifunctional abilities offers new possibilities in diagnosis, treatment and disease monitoring.

5.1 Choice of Materials for Nanotechnology

The material property of the delivery system is essentially the most important factor that governs the biocompatibility of formulation, stability and bioavailability of the drug and its clearance from the body. It is also equally important to understand the microenvironment of the target where the drug has to be delivered to achieve an effective therapeutic concentration. Design of nanosystems governed by microenvironment of the disease site results into a class of delivery systems that are popularly known as stimuli-responsive DDS. The delivery payload, route of administration and material safety profile, would also govern the components of such delivery vehicles.

A conceptual model of multifunctional nanomedicine with targeting ability, imaging capability, and drug/gene delivery in a single platform



5.2 Surface Modification to Increase Availability at Tissue and Cell Levels

A careful designing of the nanosystems will enable them to deliver the drugs successfully to the target disease through active or passive targeting. However, to do so successfully, the DDS should be available in the blood stream for longer period of time by avoiding recognition by the components of immune system, circumventing the process of opsonization and preventing subsequent clearance by the RES. The longevity of nanosystem in the circulation not only allows their deposition at the target site through EPR effect but also improves targeting ligand to interact to its receptor. Suitable surface modifications of the nanocarriers for a prolonged and sustained presence in the body have therefore garnered tremendous interest. Water-soluble polymers have been most commonly used to improve the retention time of the nanosystem in the blood and PEG is found to be most efficient in this regard. The PEG coating on the nanosystem surface provides a steric hindrance that prevents the interaction and binding of blood proteins to nanoparticle surface. The fact that RES recognition of a foreign object in the body largely depends on the binding on these plasma proteins to the surface, the sterically stabilized nanocarriers successfully escape body clearance. This property to evade the immune system is popularly known as the “stealth” effect of the polymer. PEG is an excellent choice as surface protection moiety due to its high solubility in aqueous medium, flexibility of chain length, low immunogenicity and low toxicity. Besides, it does not interfere with the biological performance of the drug loaded in the delivery vehicle. PEG therefore by far is the most studied surface modifying agent to improve the residence time of the pharmaceutically relevant nanosystems.

5.3 Image-Guided Therapy

Imaging is an indispensable component of therapy and has been routinely used in hospitals and clinics for diagnosis of diseases and defects in the body. Conventional methods such as computerized axial tomography (CAT), magnetic resonance imaging (MRI), X-Ray imaging etc. have been employed in medical science for past several decades. Therefore, it was only fitting that with the advent of nanotechnology and more specifically nano-pharmaceutics, the concept of “molecular imaging” has been envisioned. Ability to image a DDS has therefore been an integral aspect of drug delivery application since it provides a visual feature to locate the site and extend of a disease in the body.

5.4 Combination Therapeutics

Reports of multiples drug resistance (MDR) against antibacterial, antiviral, antifungal and anticancer drugs have become regularity in the previous decade. Numerous research endeavors have been applied to understand the origin of MDR and design therapeutic agents against them. However, the more we strive to overcome the medical enigmas by new drug discovery, the more complex the problem of MDR becomes. The gravity of the situation can be envisaged by a fact that the probability of MDR tuberculosis infection in acquired immunodeficiency syndrome (AIDS) patient is many folds more than a normal person. The inception of drug resistance has triggered the use of combination of drugs targeting a disease causing organism/process. The components of combination therapy may impact different independent targets, complement each other effect on the same target or bind independent of each other to give a combined effect for containment of the disease. Such combination

therapy has successfully been realized in the treatment of cancer, diabetes, bacterial and viral infections and asthma.

6. Conclusions and Future Outlook

With greater understanding of chemical and physiological barriers associated in drug delivery and advances in nanomedicine design, there is an opportunity to efficient delivery of small and macromolecular drugs to complex diseases. The nanosystems have been engineered with specific attributes such as biocompatibility, suitable size and charge, longevity in blood circulation, targeting ability and image guided therapeutics, which can deliver the drug/imaging agent to the specific site of interest, based on active and passive targeting mechanisms. These systems cannot only improve the drug delivery to the target disease, but also the resolution of detection at cellular and sub-cellular levels.

To fully realize the potential of nanosystems for delivery of contemporary therapeutics in clinical setting, it is imperative that researchers also address the material safety, scale-up and quality control issues. Scale-up and quality control becomes extremely challenging especially when dealing with nanosystem designed to carry multiple drugs, imaging agents and targeting moieties. Furthermore, in vivo fate of nanomedicine engineered using novel nanomaterials are need to be fully assessed before being used in clinical application.

RECENT DRUG ALERTS AND STATEMENTS ISSUED BY USFDA-2019

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S. No	DATE	DRUG ALERTS AND STATEMENTS
1	4/11/2019	USFDA issued warning letter to CADILA for Moraiya formulation facility in Ahmedabad.
2	28/10/2019	US FDA alerts health care professionals and patients to multiple voluntary recalls of Ranitidine
3	26/10/2019	USFDA recalls Alprazolam Tablets, USP C-IV 0.5 mg of Mylan Pharmaceuticals Inc.for Potential presence of foreign substance
4	15/10/2019	USFDA posted a warning letter to Torrent Pharmaceuticals in Ahmedabad, Gujarat, India for impurities in angiotensin II receptor blockers (ARBs) such as valsartan, losartan and irbesartan
5	11/10/2019	USFDA advised consumers not to use Rompe Pecho cough syrup
6	24/ 9 /2019	USFDA announced voluntary recall of Sandoz ranitidine capsules following detection of an impurity
7	13/9/2019	US FDA warns about rare but severe lung inflammation with Ibrance, Kisqali, and Verzenio for breast cancer
8	23/7/2019	US FDA advises patients not to use Herbal Doctor Remedies' medicines
9	26/7/2019	US FDA approved Boxed Warning about increased risk of blood clots and death with higher dose of arthritis and ulcerative colitis medicine tofacitinib (Xeljanz)
10	28/6/2019	USFDA warns patients and health care professionals not to use sterile products from Pacifico National Inc., dba AmEx Pharmacy
11	25/62019	US FDA warns consumers not to use products marketed by Kratom NC, of Wilmington, North Carolina, due to microbial

POLYMER TECHNOLOGY: A LIGNIN'S ERA

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Lignin, the second-most abundant natural polymer in plantae (second to cellulose), and often coheres with cellulose and hemicellulose, forming the main supporting structure of plants. The global increment per year of lignin via biosynthesis has been estimated to be 6 × 1014 t. Unfortunately, the complex structure of lignin makes it difficult to understand and use. Lignin, however, is regarded as a wonderful biomass chemical raw material and receives much attention in the field of materials. This is because of its varied functional group, renewability, degradability, nontoxicity, and low cost (lignin could be produced as a byproduct in paper industry). Lignin recently has been used in phenol-formaldehyde resin, polyurethane, epoxy resin, and ion exchange resin. Lignin, as a filler, also has been used to modify many kinds of rubbers, polyolefin, polyester, polyether, starch, protein, and other fossil fuel-based or biomass materials. These uses have led to many successful research and development projects for engineering plastics, adhesives, foam materials, membranes, nanofibers, hydrogels, and other new materials with great potential. Modified-materials based on film-like and nanofibrous lignin could be used as precursors to prepare carbon membranes and carbon fibers. Meanwhile, lignin and its derivates also could be used as surfactants or flocculants for oil exploitation, asphalt emulsification, dilution of oil-drilling muds, wastewater treatment, dispersion of coal water mixture or dye, water reduction or aid-grinding for concrete, controlled release of fertilizers and pesticides, antiviral, anticancer, and drug-carrying.

Table 1: The types, count, purity, and potential application of lignin

Lignin	Type world yield/109 t	Lignin purity	Potential application
Low-purity lignin	500,000	Low	Energy, refinery (splitting for carbon)
Lignosulfonate	10,000	Low-middle	Refinery (splitting for carbon), cement additives
Lignin sulfate	600	High	Pitch, refinery (splitting for carbon), cement additives, biofuel, high-quality lignin, BTX (benzene, toluene and xylene), active carbon, phenolic resin, carbon fibers, vellinine, and phenol
Organic-solvent lignin	10	High	Active carbon, phenolic resin, carbon fibers, vellinine, and phenol

			derivates
High-quality lignin	-	Extremely high	Carbon fibers, vellinine, and phenol derivates

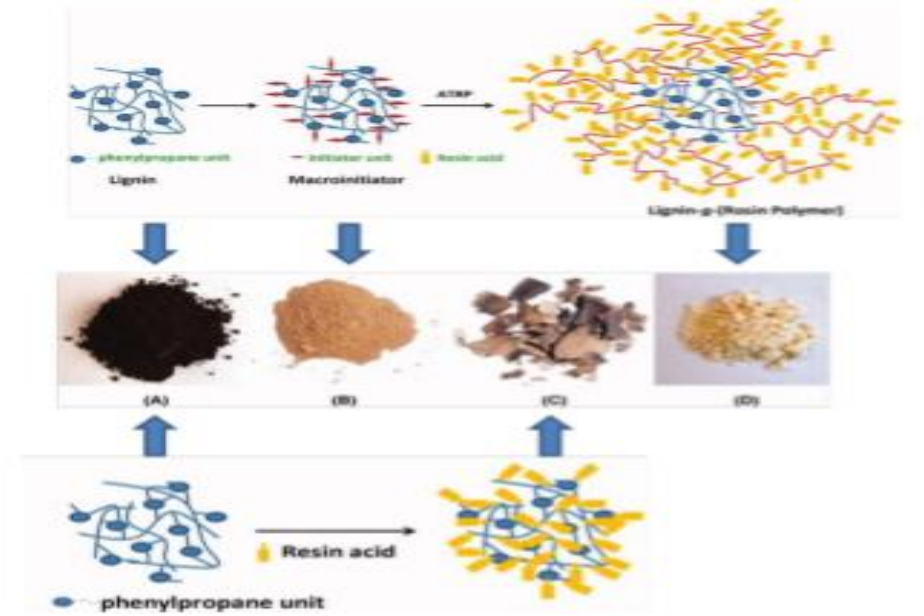

Fig. 1 The synthesis route of lignin-polyester materials, and the schematic diagram of the corresponding raw materials and products. (A) Lignin. (B) Lignin-Br. (C) Lignin-g-DA. (D) Lgema.

Pharmaceutical Applications

In the field of medicine, lignin can be used as a raw material of methyldopa (vasodilator) and dopa (Parkinson's disease drug), or to prepare antibacterial synergists, antiinflammatory agents, anticancer agents, and stimulants. Current studies focus on the antiviral or antitumor aspects that are based on lignin derivatives, such as lignosulfonate or lignin/ carbohydrate composites.

Heparin, dextran sulfate, fucoidan sulfate, and sulfated galactose carbohydrate sulfate can be used to inhibit HSV and HIV . Similarly, lignin-type carbohydrates and their sulfates also can inhibit HSV-1, HSV-2, and HIV-1 . Studies prove their structural basis for inhibiting the viruses is similar to that of sulfation heparin. The results are shown in Fig. 2.

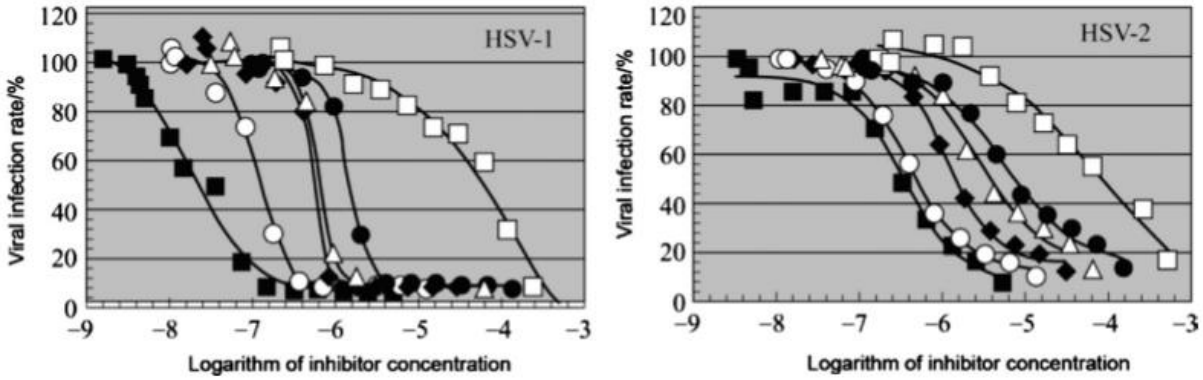

Fig. 2 The inhibiting performance of lignosulfonate on the HSV-1 invasion and HSV-2 invasion of HeLa cells.

In addition to directly using lignin derivatives to inhibit viruses and tumors, modified lignin materials also have been tried as carriers for drug delivery. The polymer film of the lignin/ starch composite can be used as a control-release carrier of ciprofloxacin hydrochloride drug. The drug control-release results of that film are shown in Fig. 3. The drug release rate increases as pH decreases. The drug release rate increases as pH decreases. At higher pH conditions, the increased swelling rate of the membrane carrier is the main reason for the decreased release rate of the drug.

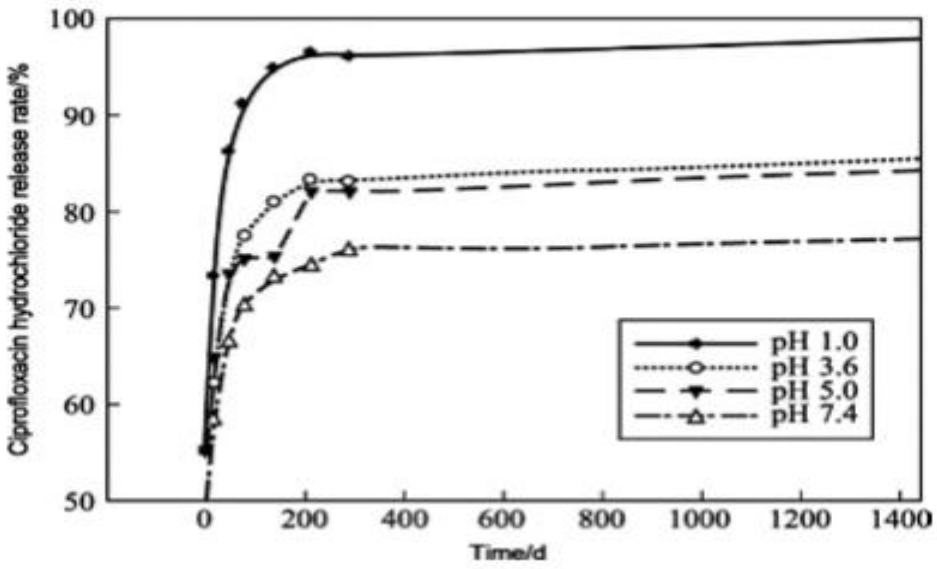

Fig. 3 The influence of pH on the release of ciprofloxacin

Lignin is also used to develop gene delivery of nano-carriers. For example, lignin nanotubes, as a gene delivery vehicle shown in Fig. 4 have been prepared by using the alumina membrane as the template. The results show that the source and separation method (such as mercaptoacetic acid, phosphoric acid, sulfuric acid, sodium hydroxide) of lignin directly affect the size, cytotoxicity, and gene transfection efficiency of the obtained lignin nanotubes.

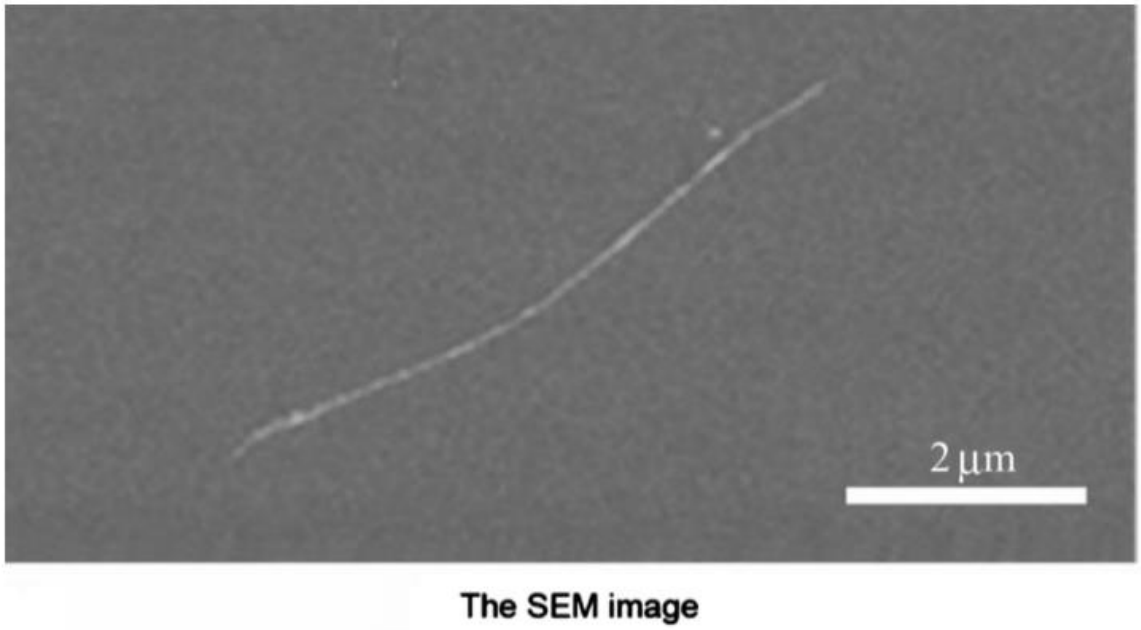

Fig. 4. The SEM image of lignin nanotubes (the lignin is extracted with NaOH).

Concluson

Although research and development based on lignin have made rapid progress, there are few actual large-scale applications of lignin, not only because of its complex multilevel structure, but also because of the lack in systematic theoretic support for its chemical modification and material-development. The breakthrough in compositing and processing of lignin-based materials, therefore, still is badly needed. Under the global concern for comprehensive use of biomass sources (to replace fossil fuelbased mass materials). Improving understanding about the structure and properties of lignin and its modified materials are conducive to increasing the application value of lignin in the field of materials, along with exploring new methods for developing high-value application based on lignin.

NANOROBOTS: THE EMERGING TOOLS IN MEDICINAL

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Nanorobotics is emerging as a demanding field dealing with miniscule things at molecular level, and it is mainly used for medical applications. Nanorobots are nano electromechanical systems designed to perform a specific task with precision at nano scale dimensions. Its advantage over conventional medicine lies on its size. The design of nanorobots is derived from biological models, specifically in the behaviour of bacteria. The various components in the nanorobots design may include on board sensors, motors, manipulators, power supplies, and molecular computers. The idea of introducing small submarines through the blood vessels has been captured in many films. But the blood at nanoscale becomes viscous and sticky fluid which does not let the submarine to drive along the vessels. Another phenomenon that would not let the submarine to travel is the Brownian movement of the molecules; the collisions between molecules are in controllable and unpredictable.

Theory of Nanorobots

Since nanorobots would be microscopic in size, it would probably be necessary for very large numbers of them to work together to perform microscopic and macroscopic tasks. These nanorobot swarms, both those which are incapable of replication (as in utility fog) and those which are capable of unconstrained replication in the natural environment (as in grey goo and its less common variants), are found in many science fiction stories, such as the Borg nanoprobes in Star Trek. The word "nanobot" (also "nanite", "nanogene", or "nanoant") is often used to indicate this fictional context and is an informal or even pejorative term to refer to the engineering concept of nanorobots. The word nanorobot is the correct technical term in the nonfictional context of serious engineering studies.

Some proponents of nanorobotics, in reaction to the grey goo scare scenarios that they earlier helped to propagate, hold the view that nanorobots capable of replication outside of a restricted factory environment do not form a necessary part of a purported productive nanotechnology, and that the process of self replication, if it were ever to be developed, could be made inherently safe. They further assert that free-foraging replicators are in fact absent from their current plans for developing and using molecular manufacturing.

Elements of Nanorobots

Carbon Nano tube, motor, bio sensors, DNA Joints

Approaches

1. Biochip:
   The joint use of nanoelectronics, photolithography, and new biomaterials, can be considered as a possible way to enable the required manufacturing technology towards nanorobots for common medical applications, such as for surgical instrumentation, diagnosis and drug delivery. Indeed, this feasible approach towards manufacturing on nanotechnology is a practice currently in use from the electronics industry. So, practical nanorobots should be integrated as nanoelectronics devices, which will allow tele-operation and advanced capabilities for medical instrumentation.
2. Nubots:
   Nubot is an abbreviation for "nucleic acid robots." Nubots are synthetic robotics devices at the nanoscale. Representative Nubots include the several DNA walkers reported by Ned Seeman's group at NYU, Niles Pierce's group at Caltech, John Reif's group at Duke University, Chengde Mao's group at Purdue, and Andrew Turberfield's group at the University of Oxford.

MICROORGANISMS AS *INVITRO* MODELS FOR MIMICKING MAMMALIAN DRUG METABOLISM

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Drug metabolism

Metabolism or biotransformation is defined as simply the structural or chemical alteration of a drug by enzymatic systems. Biotransformation is the process whereby a substance is changed from one chemical to another [transformed] by a chemical reaction within the body. Metabolism is a major determinant of the pharmacokinetic properties of most drugs and is often behind bioavailability problems, drug-drug interactions, and metabolic idiosyncrasies.

Enzymes involved in biotransformation of the drugs

The enzymes involved in biotransformation of the drugs are broadly divided into two categories, Microsomal enzymes and Non-microsomal enzymes.

Microsomal enzymes

Microsomes or vesicular fragments are organelles which are derived from rough endoplasmic reticulum and shed their ribosomes to become smooth surfaced. The microsomal enzymes are useful in catalyzing majority of drug biotransformation reactions. The microsomal enzymes are non-specific in action, can be induced or activated which metabolize only lipid soluble drugs. Ex: CYPs, FMOs, UGTs etc.

Non- Microsomal enzymes

The non-microsomal enzymes are the substances which are useful in catalyzing a few oxidative, a number of reductive, hydrolytic reactions and conjugation reactions other than glucuronidation. The non-microsomal enzymes are those which are present in soluble form in the cytoplasm and some are present mitochondria but not in the endoplasmic reticulum Eg- oxidases, esterases, amidases, conjugates. It is important to note that, the non-microsomal enzymes are soluble enzymes which mainly act on water-soluble drugs.

Products of drug metabolism

Drugs and other xenobiotics can be biotransformed in human beings to pharmacologically active metabolites which may be therapeutically / pharmacologically effective or toxic in nature. Drug metabolism is an important elimination pathway, where metabolism produces products that are more polar and therefore they can be easily excreted out of the body.

Different models used for drug metabolism studies

Preclinical screening stage is one of the important stage in development of a new therapeutic agents. The main objective of this stage is investigation of pharmacokinetic, pharmacodynamic and toxicological properties of new drug entity. Different types of both *in vitro* and *in vivo* models are used in this preclinical stage. Drug biotransformation plays a major role in investigation of therapeutic and toxic profiles of a drug molecule. After biotransformation a drug undergoes detoxification and excretion, sometimes bioactivation. Hence drug biotransformation has a major role in the early developmental stage of new drugs. Biotransformation mainly occurs in liver. The Cytochrome P450 [CYP] enzymes involve in biotransformation are mainly present in the liver. Different *in vitro* models were developed such as human CYP and UGT supersomes [baculovirus insect-cell-expressed], human liver microsomes [HLM], human liver cytosol fractions, human liver S9 fractions, liver cell lines, transgenic cell lines, liver slices, hepatocytes and isolated perfused liver. These models are used for the study of biotransformation and also in prediction of drug–drug interactions at the metabolic level.

Animal models for drug metabolism studies

Drug metabolism studies have relied on the use of model systems to produce the expected human metabolites of drugs. Generally, whole animal systems are used, especially small laboratory animal models [e.g., rat, dog, cat, guinea pig, and rabbit].

Microbial models

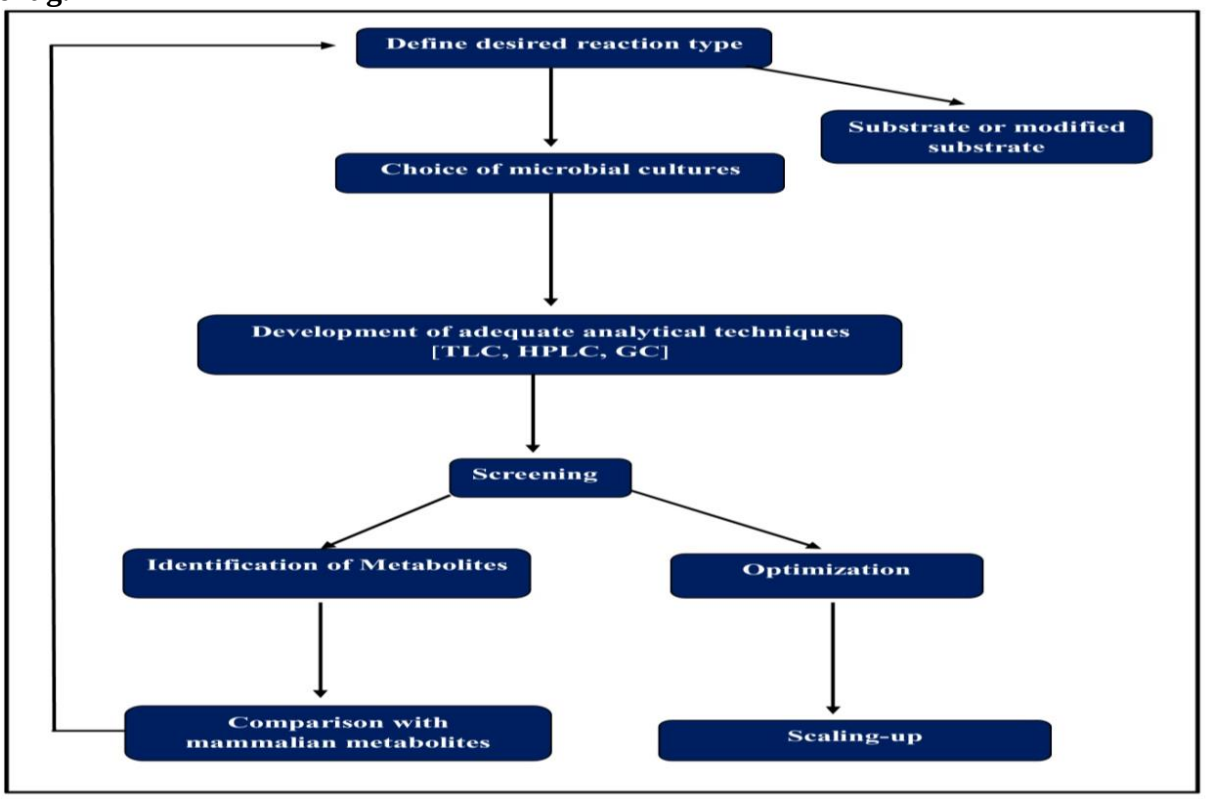
Microbial model is one of the *in vitro* model which constitute an alternative to the use of animal model and other *in vitro* models. The hydrolytic and reductive capabilities of microorganisms especially fungi have been well known for a long time and are currently used in preparative reactions concerning oxidative reactions. The classical microbial 11-α and 11-β-hydroxylation of steroids clearly parallels the same reactions in mammals. In addition, several observations on fungi reported to possess monooxygenase enzyme systems which were similar to the mammalian hepatic monoxygenases. New metabolites with high activity and less toxicity can be discovered and metabolites with new biological activities can be obtained constituting new leads for pharmacological research. The fungi, like mammals are eukaryotic organisms, are the most commonly utilized microorganisms in the biotransformation studies.

Advantages of microbial models

There are number of practical advantages with the use of microbial systems as models for drug metabolism studies such as

a. Simple culture media are easily prepared at low cost.
b. Screening for the ability of a large number of strains to metabolize the drug is a simple repetitive process, requiring only a periodical sampling of incubation media.
c. Because of the rapid rates of microbial metabolism, concentrations used [generally ranging from 0.2 to 0.5 g/l] are much higher than those employed in other cell or tissue models and, consequently, the amount of metabolites formed tends to be in the 20 to 200 mg/L range. That allows easier detection, isolation, structural identification and immediate comparison with animal metabolites.
d. Newer metabolites with a new or different activities or less toxicity can be isolated.
e. There is a possibility of predicting the most favored metabolic reactions.
f. The models can be scaled up easily for the preparation of metabolites for pharmacological and toxicological studies.
g. Microbial models can be utilized for synthetic reactions involving many steps.
h. The models can be useful in cases where stereo specificity is required.
i. Reduces the use of animals.
j. Ease of set up and manipulation.
k. More reliable and reproducible as significant species variation in small animal model made them less reliable than microorganisms.
l. Manipulation of experimental parameters of culture or incubation [induction, aeration, enzyme inhibition, etc.] can be used to control the generation of the desired metabolites.
m. Identification of active metabolites from prodrugs is facilitated and so it is the early prediction of possibly activated metabolites.
n. When new unexpected metabolites are produced, it is possible to deduce from them the likely metabolic pathways operating in animals.

General strategy for the development of a microbial model for study of metabolism of a drug.



Selection of suitable microorganisms

The selection of microorganisms is based on a number of factors including the available literature and experience. The initial goal is to identify microorganisms capable of performing desired transformation. Cultures may be isolated from soil, air or from sewage treatment facilities or may be obtained in pure form from standard culture collections.

Incubation protocol

A two stage fermentation procedure has been previously recommended for obtaining high activities during culturing. Whereas, with fungi and for initial screening studies, a one stage culture in Erlenmeyer flasks containing 50 ml of medium in a 250 ml flask is used. Preparative scale incubations are achieved in large size Erlenmeyer flasks holding several liters of medium in stirred tank or using bench top fermentors. The incubation work with fungi may start from freshly sporulated agar slants and using spore suspension for inoculation. Incubation at a specified temperature [generally 28°C for fungi and yeasts and 37°C for bacteria] for 48-72 hours in a shaker incubator [120-150 rpm] generates a culture containing large amount of biomass. Drugs are usually added to the grown cultures as solutions [1-3 vol. %] in a medium miscible, nontoxic solvent such as ethanol, acetone, dimethylformamide or dimethylsulfoxide.

Analysis of drugs and their metabolites

The development of appropriate analytical methods is essential to precede screening studies. The most commonly used analytical techniques include thin-layer chromatography [TLC], gas chromatography [GC], and high-performance liquid chromatography [HPLC] possibly coupled with mass spectrometry [MS]. The recently developed LC-NMR coupled technique appears to be especially suitable for analysis of relatively large quantities that can be produced using microbial models.

DEPARTMENT OF PHARMACOLOGY

NIPAH VIRUS-PRESENT STATUS AND ITS MANAGEMENT

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Nipah virus is a zoonotic virus. It is transmitted from animals to humans and can also be transmitted through contaminated food or directly between people. In infected people, it causes a range of illnesses from asymptomatic (subclinical) infection to acute respiratory illness and fatal encephalitis. The virus can also cause severe disease in animals such as pigs, resulting in significant economic losses for farmers. Although Nipah virus has caused only a few known outbreaks in Asia, it infects a wide range of animals and causes severe disease and death in people, making it a public health concern. Nipah virus infection in humans causes a range of clinical presentations, from asymptomatic infection to acute respiratory infection and fatal encephalitis. Nipah virus was first recognized in 1999 during an outbreak among pig farmers in, Malaysia. No new outbreaks have been reported in Malaysia since 1999. It was also recognized in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. The disease has also been identified periodically in eastern India.

During the first recognized outbreak in Malaysia, which also affected Singapore, most human infections resulted from direct contact with sick pigs or their contaminated tissues. Transmission is thought to have occurred via unprotected exposure to secretions from the pigs, or unprotected contact with the tissue of a sick animal. In subsequent outbreaks in Bangladesh and India, consumption of fruits or fruit products (such as raw date palm juice) contaminated with urine or saliva from infected fruit bats was the most likely source of infection. There are currently no studies on viral persistence in bodily fluids or the environment including fruits.

Human-to-human transmission of Nipah virus has also been reported among family and care givers of infected patients. Human infections range from asymptomatic infection to acute respiratory infection (mild, severe), and fatal encephalitis.

Infected people initially develop symptoms including fever, headaches, muscle pain, vomiting and sore throat. This can be followed by dizziness, drowsiness, altered consciousness, and neurological signs that indicate acute encephalitis. Some people can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours. Initial signs and symptoms of Nipah virus infection are nonspecific, and the diagnosis is often not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection, effective and timely infection control measures, and outbreak response activities. Nipah virus infection can be diagnosed with clinical history during the acute and convalescent phase of the disease. The main tests used are real time polymerase chain reaction (RT-PCR) from bodily fluids and antibody detection via enzyme-

linked immunosorbent assay (ELISA). There are currently no drugs or vaccines specific for Nipah virus infection although WHO has identified Nipah as a priority disease for the WHO Research and Development Blueprint. Intensive supportive care is recommended to treat severe respiratory and neurologic complications. Favipiravir, Remdesivir and a monoclonal antibody m102.4 are under investigation. Fruit bats of the family *Pteropodidae* – particularly species belonging to the *Pteropus* genus – are the natural hosts for Nipah virus. There is no apparent disease in fruit bats.

Outbreaks of the Nipah virus in pigs and other domestic animals such as horses, goats, sheep, cats and dogs were first reported during the initial Malaysian outbreak in 1999. The virus is highly contagious in pigs. Pigs are infectious during the incubation period, which lasts from 4 to 14 days. Currently, there are no vaccines available against Nipah virus. Based on the experience gained during the outbreak of Nipah involving pig farms in 1999, routine and thorough cleaning and disinfection of pig farms with appropriate detergents may be effective in preventing infection. Researchers developed a novel recombinant vaccine called NIPRAB that shows robust immunization against Nipah virus in animal models and may be effective against other viruses in the same family and research is going on. In the absence of a vaccine, the only way to reduce or prevent infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the Nipah virus. Bat-to-human transmission, animal-to-human transmission and human-to-human transmission should be controlled.

The risk of international transmission via fruits or fruit products such as raw date palm juice contaminated with urine or saliva from infected fruit bats can be prevented by washing them thoroughly and peeling them before consumption. Fruit with signs of bat bites should be discarded.

ANXIOLYTIC AND ANTI-DEPRESSANT ACTIVITIES OF ETHANOLIC EXTRACTS OF *JASMINUM SAMBAC, CHAMOMILLA CAPITULA, LILIUM CANDIDUM, SORGHUM HALPENSE* FLOWERS

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Abstract

The objective of the present study to evaluate the anxiolytic and antidepressant activities of ethanolic extract of *Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense* Flowers using elevated plus maze, actophotometer, Froced swim test and Tail suspension test in mice. Albino mice were treated at a dose of 200 and 400 mg/kg I.P and behavior was observed on these models. Results in elevated plus-maze have showed that both the number of open arm entries and time spent in the open arms were significantly increased in case of the animals treated with combination of flower extracts that is EEJSLC compare to the animals treated individuals i.e. EEJs, EELc, EESh and EECc thereby producing anti-anxiety activity where as Locomotors activity is considered as an index of alertness, and the spontaneous decrease in basal activity score implicates the reduction of anxiety. Such types of effect can be found in the case of sedatives. The anti-depressant activity in forced swim test was observed that EESh1, EESh2 and EECc2 has exhibited significant reduction in immobility time when compared to control in dose dependent manner. Whereas, tail suspension test was significantly increases the immobility time. The magnitude of the antidepressant effects of EEJSLC2 shows same significant effect as that of standard drug Diazepam 10 mg/kg i.p. whereas EEJSLC1 is highly significant.

Keywords: *Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense* Flowers, elevated plus maze, actophotometer, Froced swim test and Tail suspension test.

Introduction

According to the world health report approximately 450 million people suffer from a mental or behavioural disorder, but only a small number them receive even the foremost basic treatment, this accounts for 12.3% of the global burden of disease and will increase to 15% by 2020. Herbal drugs are widely used for the treatment of various diseases. Although herbal drugs often contain highly active pharmacological compounds but much importance is not given to their safety evaluation, may be due to a popular notion “anything herbal is safe.” Lately, with recent increasing interest in traditional or herbal drugs for the prevention and treatment of various disorders, there is also increasing concern about the safety of traditional, herbal product based medicines. The current research also focuses on the extraction and CNS activities of peels of citrus fruits which are easily available at zero cost thus decreasing the cost for production. The following fruits have been targeted in the present study.

Jasminum sambac. Linn (Oleaceae) is commonly known as Jasmine. It is a well known glabrous twining shrub widely grown in gardens throughout India. The flower is acrid and bitter taste. It is useful in treating diseases of the mouth and teeth, especially for toothache. The J. sambac flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of J. sambac are useful to women when brewed as a tonic as it

aids in preventing breast cancer and stopping uterine bleeding. It is widely used in the Ayurveda, as an antiulcerative, anti cancer, antileprotic, skin diseases and wound healing.

Chamomile capitula L. (family Asteraceae), popularly known as Chamomile is a reputed medicinal and aromatic plant used in both traditional and modern system of medicine. It is an ingredient of several traditional, Unani and Homeopathy medicinal preparations. The capitula of chamomile contain natural oil, known as 'blue oil' (essential oil). The essential oil of chamomile has anti-inflammatory and softening effect and is useful in the treatment of gastric colic enteralagia, gastritis, bloating, inflammation and respiratory tract.

Lilium candidum L. (Liliaceae), the so called "white Madonna lily", is well known in folk medicine for the treatment of burns, ulcers, inflammations and for healing wounds. Lilium candidum L. extract contains various biologically active compounds. As the antimutagenic activity of natural compounds often correlates with antioxidant effects and contents of phytochemical substances from the flavonoids group, our hypothesis is that the LC extract, which is rich in flavonoids and with pronounced antioxidant activity, could possess bioprotective potential.

Sorghum halepense L. (Poaceae) is adapted to a wide variety if habitats including open forests, old fields, ditches and wetlands. It occurs extensively along irrigated canals and at the edges of irrigated fields; its general distribution in these areas is the result of water movement of the seeds, which readily fall from the head when mature. Select varieties of sorghum have considerably high concentrations of phenolic compounds and antioxidant capacities that are located primarily in the bran fraction of the grain. Flavonoids, phenolic acids and tannins are three phenolic categories found in sorghum.

Numerous bioactive compounds such as flavonoids, Saponins, Phenolic and tannins have been isolated from flowers of this four extracts. Some of these bioactive compounds have been worked out for one or the other medicinal attributes. But till date, the CNS activities of this flowers extracts have not been scientifically evaluated. Hence, in the present study, the effect of this four flower extracts at a dose of 200 and 400mg/kg body wt on antidepressant and anxiolytic activities has been studied.

Materials and Methods

Preparation of plant extracts: Fresh flowers of *Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense* were collected and dried under shade. The extracts used were prepared by taking 20gms of finely coarsely powdered was taken in a 250ml beaker containing 200ml of ethanol. The contents were mixed well and then the mixture was boiled upto 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Phytochemical analysis of the extracts

The extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytoconstituents such as alkaloids, terpenoids, saponoins flavonoids, phenols and tannins.

Pharmacological evaluation

Preparation of extracts: The ethanolic extracts of *Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, and Sorghum Helpense* suspended in water in presence of 3%v/v Tween-80 solution. All the drugs were administered I.P for experimental purpose. Each time

preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

Acute Oral Toxicity: The acute oral toxicity of ethanolic extracts of *Lilium Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense and their combinations* was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 2000 mg/kg of body weight.

Procedure for Antianxiety Activity

Elevated plus maze (EPM) model

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted. The procedure was conducted preferably in a sound attenuated environment.

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Turn on the equipment (check & make sure that all the photocells are working for accurate recording) and placed individually each rat in the activity cage for 10 minutes. Note the basal activity score of all the animals. Inject the drug diazepam (Dose: 5 mg/kg, i.p; make a stock solution containing 0.5 mg/ml of the drug & inject 1 ml/100 g body wt of mouse), and after 30 mins re-test each mouse for activity scores for 10 mins. Note the difference in the activity, before & after chlorpromazine. Calculate percent decrease in motor activity.

Procedure for Antidepressant Activity

Despair Swim Test

Apparatus For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10cm, at 25 ± 2°C. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming[17].

Tail suspension test

Tail suspension test was performed based on the method prescribed[18]. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remain motionless[18].

Results and Discussion

Preliminary Phytochemical Screening:

Phytochemical investigation of ethanolic extracts of *Lilium Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, and Sorghum Helpense* revealed the presence of alkaloids, tannins, saponins, terpenoids and flavonoids as secondary metabolites.

Acute toxicity testing

Acute toxicity studies revealed that the ethanolic extracts of *Lilium Jasminum Sambac, Chamomilla Capitula, Lilium Candidum, Sorghum Helpense* were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

Evaluation of Antianxiety Activity

Elevated Plus Maze Test

Anxiolytic property of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Elevated plus maze experiment.

In elevated plus-maze test (EPM), the extracts of EEJs1, EELc1, EEJSLC1 and EEJSLC2. Significantly increased the number of entries and time spent into the open arm. The magnitude of the antianxiety effects of ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* was compared with the standard drug diazepam 10 mg/kg i.p.

Table:3 - Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on Elevated Plus Maze test in rats.

S.No	Groups	Dose (mg/kg)	No.of entries		Average time spent (sec)		No.of rearings
			(O)	(C)	(O)	(C)	
1.	Normal control	-	1	7	9	291	7
2.	Diazepam	10	2	4	25	275	9
3.	EEJs1	200	3	3	37	263	5
4.	EEJs2	400	2	5	19	281	10
5.	EESh1	200	3	4	28	272	7
6.	EESh2	400	2	5	18	282	9
7.	EELc1	200	3	4	22	278	6
8.	EELc2	400	4	2	47	253	5
9.	EECc1	200	2	4	22	278	6
10.	EECc2	400	1	3	18	282	10

11.	**EEJSLC1**	**200**	2	5	38	262	12
12.	**EEJSLC2**	**400**	3	6	44	256	6

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates that no.of entries were increased in open arms.

Actophotometer Test

Anxiolytic property of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Actophotometer experiment.

The percentage of reduction in locomotor activity with diazepam (10 mg/kg i.p) after 1 hour is 91.0 % i.e. there is highly significant (P<0.05) decrease in locomotor activity compare to control, where as dose of (200 and 400mg/kg i.p) showed dose dependent decrease in locomotor activityis EEJs1, EESh1, EELc1, EELc2, EEJSLC1 and EEJSLC2 that is 78.3%, 77.9%, 76.6%, 72.2%, 82.5% and 79.5% respectively when compared to standard. The values are highly significant (P<0.05) (Table No :4).

Table No: 8.12. Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* on Locomotor activity.

S.No	Groups	Dose (mg/kg)	Locomotor activity (scores) in 10 min		
			Before	After	%change in activity
1.	**Control**	**-**	245	--	---
2.	**Diazepam**	**10**	270	85	68.5
3.	**EEJs1**	**200**	365	79	78.3
4.	**EEJs2**	**400**	286	88	69.2
5.	**EESh1**	**200**	236	52	77.9
6.	**EESh2**	**400**	233	79	66.0
7.	**EELc1**	**200**	240	56	76.6
8.	**EELc2**	**400**	231	64	72.2
9.	**EECc1**	**200**	281	94	66.5
10.	**EECc2**	**400**	243	89	63.3
11.	**EEJSLC1**	**200**	240	42	82.5
12.	**EEJSLC2**	**400**	274	56	79.5

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in locomotar activity.

Evaluation of Antidepressant Activity

Forced Swim Test

Antidepresssant activity of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Forced Swim Test experiment.

The anti-depressant activity of various extracts and their combination was assessed using Forced Swimming Test in Swiss albino rats were illustrated in Table No: 1. it was observed that EESh1, EESh2 and EECc2 have exhibited significant reduction in immobility time when compared to control in dose dependent manner. Similarly, the animals treated with diazepam (10mg/kg) as expected showed significant decrease in immobility time.

Table No: 1 Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on Forced swim Test in mice.

S.No	Group	Dose(i.p; mg/kg)	Immobility period		% change in activity
			Before	After	
1.	Control	-	134	--	---
2.	Diazepam	10	185	62	66.48%
3.	EEJs1	200	179	67	62.6%
4.	EEJs2	400	305	195	36.06%
5.	EESh1	200	288	75	73.95%
6.	EESh2	400	293	82	72.01%
7.	EELc1	200	312	96	69.23%
8.	EELc2	400	259	78	69.88%
9.	EECc1	200	274	99	63.86%
10.	EECc2	400	281	76	72.95%
9.	EEJSLC1	200	186	60	67.74%
10.	EEJSLC2	400	198	65	66.66%

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in motar activity.

Tail Suspension Test

Antidepresssant activity of ethanolic extract of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied at a dose of 200 and 400 mg/Kg by using Forced Swim Test experiment.

In tail suspension test, the ethanolic extracts of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* at a dose of 200 and 400mg/kg i.p. significantly increases the immobility time. The magnitude of the antidepressant effects of EEJSLC2 shows same significant effect as that of standard drug Diazepam 10 mg/kg i.p. whereas EEJSLC1 is highly significant. (Table 2).

Table No: 8.14 Effect of Ethanolic extracts of *J.Sambac, S.halpense, L.Candidum and C.Capitula and Combination of four flowers* (200 and 400 mg/kg) on tail suspension in mice.

S.No	Treatment	Dose (mg/kg)	Duration of immobility		%change in activity
			Before	After	
1.	Control	-	40	-----	------
2.	Diazepam	10	20	120	83.33%
3.	EEJs1	200	40	180	77.8%
4.	EEJs2	400	54	167	67.7%
5.	EESh1	200	64	196	67.3%
6.	EESh2	400	25	173	85.5%
7.	EELc1	200	32	148	78.3%
8.	EELc2	400	54	152	64.4%
9.	EECc1	200	67	146	54.1%
10.	EECc2	400	48	137	64.9%
11.	EEJSL1	200	20	158	87.3%
12.	EEJSL2	400	28	162	82.7%

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in motar activity.

Conclusion

The study was performed to find out the beneficial effects of different extracts of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination* were studied for different activities such as anti-depressant and anti-anxiety activities. The results reveal that the plant has beneficial effects on these activities.

Preliminary Phytochemical Screening:

In current scenario, flowers are the potent sources of medicines used in the treatment of various disease and disorders. Since, flowers are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of flowers of *J.Sambac, S.halpense, L.Candidum and C.Capitula and their Combination*. The Phytochemical evaluation has revealed the presence of alkaloids, terpenoids, saponins, flavonoids, phenols and tannins.

Anti-Anxiety Activity

Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals. Among the extracts tested, maximum anxiolytic activity was observed in the ethanol at the dose of 200 mg/kg which was at par with that of diazepam as is evident from statistical equivalence between the results of this dose and that manifested by diazepam. However, the activity decreased at higher doses, which might be due to sedation. Phytochemical screening showed presence of alkaloids in ethanol extract of *J.Sambac, S.halpense, L.Candidum and C.Capitula*. Alkaloids were subjected to biological evaluation for anti-anxiety activity in rats using EPM apparatus and Actophotometer.

Therefore, this plant merits further attention. Search on the most active principle as well as elucidation of the exact mechanism of its action is needed. Thus, we conclude that ethanol extract of *J.Sambac, S.halpense, L.Candidum and C.Capitula* flowers possess anti-anxiety activity and studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

Anti-Depressant Activity

Many researchers showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and steroids were found to ligands for the GABAA receptors in the central nervous system; which led to the assumption that they can act as benzodiazepine like molecules. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. It has been argued that TST (Tail Suspension Test) is less stressful than FST (Forced swim test) and has greater pharmacological sensitivity. Flavonoids present in this extracts may be facilitating monoaminergic transmission there by producing antidepressant effects. However, the activity decreased at higher doses, which might be due to sedation. Phytochemical screening showed presence of saponins in ethanol extract of *J.Sambac, S.halepense, L.Candidum and C.Capitula*. Saponins were subjected to biological evaluation for anti-depressant activity in mice using FST and TST.

RECENT ADVANCES IN PHARMACOLOGICAL RESEARCH– LATEST ARTICLE

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Challenges and Opportunities for Childhood Cancer Drug Development: Cancer in children is rare with approximately 15,700 new cases diagnosed annually. Through use of multimodality therapy (surgery, radiation therapy, and aggressive chemotherapy), 70% of patients will be "cured" of their disease, and 5-year event-free survival exceeds 80%. However, for patients surviving their malignancy, therapy-related long-term adverse effects are severe, with an estimated 50% having chronic life-threatening toxicities related to therapy in their fourth or fifth decade of life. While overall intensive therapy with cytotoxic agents continues to reduce cancer-related mortality, new understanding of the molecular etiology of many childhood cancers offers an opportunity to redirect efforts to develop effective, less genotoxic therapeutic options, including agents that target oncogenic drivers directly, and the potential for use of agents that target the tumor microenvironment and immune-directed therapies. However, for many high-risk cancers, significant challenges remain.

Targeting Foam Cell Formation in Atherosclerosis: Therapeutic Potential of Natural Products: Foam cell formation and further accumulation in the sub endothelial space of the vascular wall is a hallmark of atherosclerotic lesions. Targeting foam cell formation in the atherosclerotic lesions can be a promising approach to treat and prevent atherosclerosis. The formation of foam cells is determined by the balanced effects of three major interrelated biologic processes, including lipid uptake, cholesterol esterification, and cholesterol efflux. Natural products are a promising source for new lead structures. Multiple natural products and pharmaceutical agents can inhibit foam cell formation and thus exhibit antiatherosclerotic capacity by suppressing lipid uptake, cholesterol esterification, and/or promoting cholesterol ester hydrolysis and cholesterol efflux. This review summarizes recent findings on these three biologic processes and natural products with demonstrated potential to target such processes. Discussed also are potential future directions for studying the mechanisms of foam cell formation and the development of foam cell-targeted therapeutic strategies.

Harnessing Ion-Binding Sites for GPCR Pharmacology:

Endogenous ions play important roles in the function and pharmacology of G-protein coupled receptors (GPCRs). Historically the evidence for ionic modulation of GPCR function dates to 1973 with studies of opioid receptors, where it was demonstrated that physiologic concentrations of sodium allosterically attenuated agonist binding. This Na^+-selective effect was distinct from effects of other monovalent and divalent cations, with the latter usually counteracting sodium's negative allosteric modulation of binding. Since then, numerous studies documenting the effects of mono- and divalent ions on GPCR function have been published. While ions can act selectively and nonselective at many sites in different receptors, the discovery of the conserved sodium ion site in class A GPCR structures in 2012 revealed the unique nature of Na^+ site, which has emerged as a near-universal site for allosteric modulation of class A GPCR structure and function.

Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure: Despite the success of renin-angiotensin system (RAS) blockade by angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT_1R) blockers, current therapies for hypertension and related cardiovascular diseases are still inadequate. Identification of additional components of the RAS and associated vasoactive pathways, as well as new structural and functional insights into established targets, have led to novel therapeutic approaches with the potential to provide improved cardiovascular protection and better blood pressure control and/or reduced adverse side effects. The simultaneous modulation of several neurohumoral mediators in key interconnected blood pressure–regulating pathways has been an attractive approach to improve treatment efficacy, and several novel approaches involve combination therapy or dual-acting agents. In addition, increased understanding of the complexity of the RAS has led to novel approaches aimed at up regulating the ACE2/angiotensin-(1-7)/Mas axis to counter-regulate the harmful effects of the ACE/angiotensin II/angiotensin III/AT_1R axis. These advances have opened new avenues for the development of novel drugs targeting the RAS to better treat hypertension and heart failure.

The Purinergic System as a Pharmacological Target for the Treatment of Immune-Mediated Inflammatory Diseases: Immune-mediated inflammatory diseases (IMIDs) encompass a wide range of seemingly unrelated conditions, such as multiple sclerosis, rheumatoid arthritis, psoriasis, inflammatory bowel diseases, asthma, chronic obstructive pulmonary disease, and systemic lupus erythematosus. Despite differing etiologies, these diseases share common inflammatory pathways, which lead to damage in primary target organs and frequently to a plethora of systemic effects as well. The purinergic signaling complex comprising extracellular nucleotides and nucleosides and their receptors, the P2 and P1 purinergic receptors, respectively, as well as catabolic enzymes and nucleoside transporters is a major regulatory system in the body. The purinergic signaling complex can regulate the development and course of IMIDs. Here we provide a comprehensive review on the role of purinergic signaling in controlling immunity, inflammation, and organ function in IMIDs.

Biased Receptor Signaling in Drug Discovery: A great deal of experimental evidence suggests that ligands can stabilize different receptor active states that go on to interact with cellular signaling proteins to form a range of different complexes in varying quantities. In pleiotropically linked receptor systems, this leads to selective activation of some signaling pathways at the expense of others (biased signaling). Although this is a fairly newly discovered phenomenon, theoretical and experimental data suggest that it is a ubiquitous behavior of ligands and receptors and to be expected. Biased signaling is simple to detect in vitro and there are numerous methods to quantify the effect with scales that can be used to optimize this activity in structure-activity medicinal chemistry studies. At present, the major hurdle in the application of this mechanism to therapeutics is the translation of in vitro bias to in vivo effect; this is because of the numerous factors that can modify measures of bias in natural physiologic systems. In spite of this, biased signaling still has the potential to justify revisiting of receptor targets previously thought to be intractable and also furnishes the means to pursue targets previously thought to be forbidden due to deleterious physiology (as these may be eliminated through biased signaling).

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *TAGETES ERECTA* LEAVES ON CARBON-TETRACHLORIDE INDUCED HEPATOTOXIC RATS

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Abstract

The present study was conducted to evaluate the hepatoprotective effects of methanolic leaf extract of *Tragetes erecta* on carbon tetrachloride (CCl4) induced liver damage in *albino* rats. Wistar *albino* rats weighing around 180-200g were used. Toxicity was induced by using 30% CCl4 suspended in olive oil (1.0 ml/kg body wt intraperitoneal) after every 72 hrs for 3doses. The methanolic leaf extract at a dose of 250mg/kg was administered orally by intragastric tube for 10 days. Blood and liver tissue were collected for the assessment of serum marker enzymes such as ALT, AST and ALP. The liver tissue was used for histopathological assessment.

Keywords: *Tragetes erecta*, CCl4, Alkaline phosphatase (ALP), Alanine amino transaminase (ALT), Asparate aminotrasaminase (AST).

Introduction

The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in adequate, so far not yet any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects. Therefore, that is an essential research about suitable herbal drugs that could replace the chemical ones. Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. However, no effective treatment that delays disease progression and complications has yet been found. Several recent studies suggest that traditional herbs and micronutrients such as carotenoids and selenium may be useful for this purpose.

Carbon tetrachloride CCl4 is widely used for experimental induction of liver damage. The principle cause of carbon tetrachloride (CCl4) is induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals. Various medicinal plants have been used to treat for various diseases in all over the world. Nowadays, Indian medicinal plants are belonging to about 40 families were investigated as liver protective drugs. *Tagetes erecta* commonly known as “Marigold” is a plant belonging to the family Asteraceae. It has been found in many countries in Asia, America and Africa. The roots, stems, leaves and whole plant of *Tagetes erecta* are used in the treatment of jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhoea, etc. The leaves of this plant are widely used in Indian folklore medicine for reducing the amount of sugar in urine of diabetes patients suffering from diabetes mellitus. Literature suggests that the use of this plant is in the treatment of diabetes due its highest phenolic and flavonoid contents along with its anti oxidant activity. The crude hydromethanolic extract of the leaves of *Tagetes erecta* has been reported for its xanthine oxidase inhibitory and hypouricaemic activities.

Materials and Methods

Plant material

The *Tagetes erecta* (Family: Asteraceae) leaves were collected during Jan – Feb of 2013 in and around Kodada, Telangana were authenticated by department of botany. The voucher specimens were kept in the department of botany.

Plant extracts preparation:

The shade dried plant materials of *Tagetes erecta*, leaves were powdered separately in an electrical blender and stored at 5°C until further use. The dried plant powder was taken separately and subjected to soxhelet extraction with petroleum ether and methanol respectively. The extract was evaporated to get concentrated semi solid material. It was used for the experiment work.

Animal:

Wistar *albino* rats weighing 180-200g were collected. Animals were housed in clean polypropylene cages with 12±1 hr light/dark schedule and fed with normal pellet (Hindustan Lever Ltd., Bangalore., India) rat chow diet and water. The Institutional Animals Ethics Committee (IAEC) approved the study protocol.

Experimental design:

The rats were randomly divided into 4 groups of 6 rats each.

Group I: Animals served as normal control and received distilled water (1ml/kg body wt.,) orally for 10days.

Group II: Animals received olive oil 1ml/kg body wt i.p after every 72 hrs 3 doses.

Group III: Animals constituted the hepatotoxic group, which received 30% CCl4 in olive oil (1 ml/kg body wt i.p) after every 72 hrs 3 doses.

Group IV: CCl4 treated groups administered with *Tagetes erecta* L., methanolic leaf extracts orally 250 mg/kg body wt for 10 days to the CCl4 induced animal.

Estimation of biochemical parameters:

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood were collected and serum was separated for biochemical analysis. Liver marker enzymes such as Asparate amino transaminase (AST), Alanine amino transaminase (ALT) and Alkaline phosphatase (ALP) were estimated 12, 13. The liver tissue was excised in 10% neutral buffered formalin for histopathological studies 14.

Histopathological studies:

Anatomy of the liver was studied immediately after sacrificing the animals. A small portion was fixed in 10% neutral buffered formalin as described by Luna 14. Thin sections of 4-5 µm were taken, stained with Haematoxylin and Eosin and histology was studied. The results were expressed in Mean ± SEM, Student's't' test was used for statistical significance between groups.

Results

The results showed in the table - 1 exhibit the significant hepatoprotective effects of the plant *Tagetes erecta*. The levels of serum Aspartate amino transaminase (AST), Alanine amino transaminase (ALT) and Alkaline Phosphate (ALP) were taken as an index for hepatotoxicity induced by CCl4. The levels of AST, ALT and ALP were analyzed in serum samples of different groups of *albino* rats shown in table 1. Serum marker enzymes such as ALT, AST and ALP were analyzed for the control and experimental animals. In the group II (control) olive oil treated animals, showed the level of marker enzymes were not significantly elevated when compared to the normal group I animals. The level of marker enzymes of group III CCl4 induced animals were

Significantly increased (P<0.05) when compared to the normal and control animals. But there was a significant decrease of the enzyme level (P<0.001) in the methanolic leaf extract of *Tagetes erecta* L., treated animals (group IV). Carbon tetrachloride is reported to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of serum biochemical parameters like ALT, AST, and ALP. Histopathological studies of the liver section of control and experimental animals were shown in (fig.1- 4), was carried out to test the hepatoprotective effect of the methanolic leaf extracts of *Tagetes erecta*. The fig-1 shows the liver section of Group 1 (normal Control) animals, which has normal architecture, where the central veins, portal tracts, hepatocytes and sinusoids appear normal. The lobular unit is also well-identified .Fig-2 shows Group-II (olive oil control) animals liver section has no significant pathological changes when compared to the normal. Group-III (CCl4 induced)animals liver section (fig-3) showed the damage of the liver cells .There are extensive areas of patchy and confluent hepatocyte necrosis and lobular inflammation, Sinusoidal spaces are flooded with inflammatory cells and RBC's . The methanolic leaf extracts (*Tagetes erecta*) treated animals of Group-IV: showed complete reversal of toxic effects in the liver cells (fig-4) No necrosis seen. The central vein and portal triads appear normal. Some of the hepatocytes showed binucleation suggesting regenerative activity with feathery degeneration of hepatocytes. The improved histology of the liver as seen in histopathological observation on animals treated with the plant material as compared to that seen in animals administered only CCl4 indicated in possibility of the plant material being able to induce accelerated regeneration of the liver.

Table 1: Effect of *Tagetes erecta* methanolic leaf extract on liver marker enzymes in normal and experimental animals

Parameters	**Normal (Group I)**	**Olive oil (Group II)**	**CCl4 induced (Group III) (250 mg/kg)**	***Tagetes erecta* treated (Group IV)**
ALT(Units/ml)	46.20±6.22	49.20±2.09	119.20±10.79**	62.73±3.91**
AST (Units/ml)	54.20±5.02	56.10±2.25	172.05±11.0**	48.51±1.90**
ALP (KA Units)	51.10±8.12	52.60±2.61	104.63±7.69**	58.57±9.7**

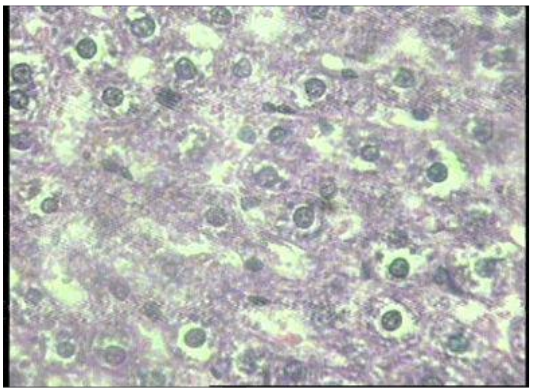

Fig-1: Normal hepatic cells

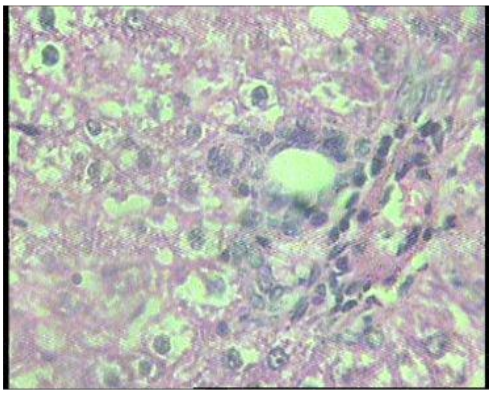

Fig-2: Olive oil treated hepatic cells

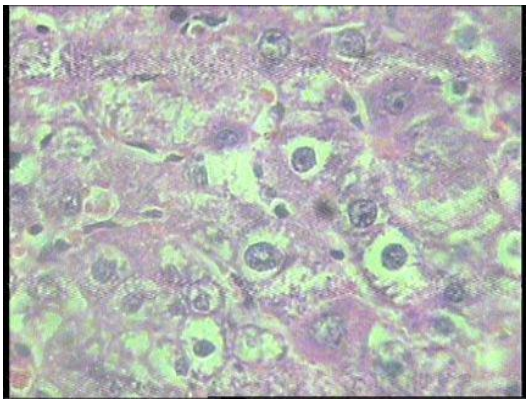

Fig-3: CCl4 induced damaged hepatic cells cells

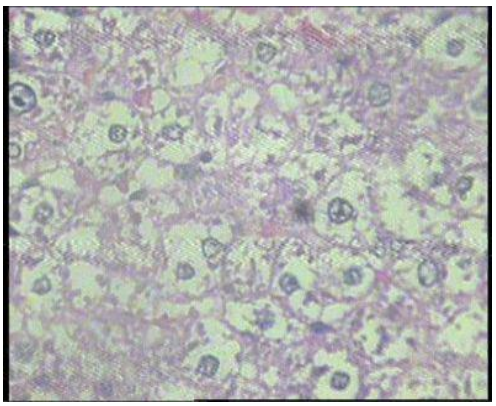

Fig-4: *Tagetes erecta* treated hepatic

Discussion

This study was under taken to demonstrate the protective ability of aqueous leaf extracts of *Tagetes erecta* on liver damage induced by CCl4 and the toxic effects of the similar doses in rats. The damage of the liver caused by CCl4 was evident by the alteration in serum marker enzymes concentration beside the clinical signs and histopathology. The use of aqueous leaf extracts of *Tagetes erecta* protects the liver from damage by CCl4 as evident by improved histologic picture and biochemical markers of liver damage. The mechanism of the hepatoprotective action of the plant is uncertain but may be related to the ability of the plant to inhibit lipid peroxidation in the liver. The CCl4 induced hepatotoxicity produced in rats leading to hepatic injury triggers the generation of toxic radicals, which can be masked by using a correct antioxidant in adequate amount. The presence of flavonoids, tannins, saponins and terpenoids in *Tagetes erecta* explain its role in hepatoprotection by inhibiting the free radicals mediated damage 15 claimed that flavonoids, triterpens and tannin were antioxidant agent and may interfere with free radicals formation 17 stated that hepatoprotective activities of certain flavonoids are known. The haemorrhage caused by CCl4 in the liver was minimized by use of plant extract, as flavonoids are known to be vasculo protector. Based on results obtained it can be concluded that the aqueous leaf extract of *Tagetes erecta* leaves seems to possess hepatoprotective activity in *albino* rats. Further studies are needed to evaluate the potential usefulness of this extract in clinical conditions associated with liver damage. In conclusion, the results of the present study indicated that under the present experimental conditions, aqueous leaf extract of *Tagetes erecta* L. Showed hepatoprotective effects against carbon tetrachloride induced liver damage in albino rats.

Conclusion

In conclusion, the results of the present study indicated that under the present experimental conditions, methanolic leaf extract of *Tagetes erecta* L. Showed hepatoprotective effects against carbon tetrachloride induced liver damage in albino rats. The haemorrhage caused by CCl4 in the liver was minimized by use of plant extract, as flavonoids are known to be vasculo protector. Further studies are needed to evaluate the potential usefulness of this extract in clinical conditions associated with liver damage.

DEPARTMENT OF PHARMACEUTICAL ANALYSIS

PIONEERING BIOPLASTIC COMPANIES IN INDIA BRING ABOUT A POSITIVE CHANGE

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Petroleum-based plastics are the third highest used product extracted from petroleum. India has become one of the biggest centers of plastic usage with over 15,000 tons of plastic waste generated every year, of which only 60% is re-processed. Countries all over the globe have begun to take steps on curbing its usage. Bangladesh has prohibited plastic bags countrywide, Ireland has imposed a tax on plastic bags, while the UK and other European countries are contemplating about taxing them as well. Comparative Advantage of Bioplastics

There are a few alternatives to plastics that are gaining attention at a global level. Bioplastics is one such eco-friendly alternative to plastics, which could be an excellent replacement since their manufacturing results in fewer emissions of greenhouse gasses. Unlike plastics, bio-plastics are made from organic biomass sources such as corn starch and sugarcane.

Popular Variants of Bioplastics

Unknowing to us, Bioplastics have been around for decades now with a notable historical usage in the Model T automotive parts that were designed by Henry Ford from corn starch and soybean oil ingredients. However, it needs to be noted that not all bioplastics are completely biodegradable. Some biological-based products can biodegrade in municipal composting facilities, or aquatic and landfill environments; others can only biodegrade in very specific environments, while some will not biodegrade at all.

There are two variants that have become more popular and have gained maximum attraction:

- Polylactic acid (PLA)
- Poly hydroxyl alkanoate

Production of bioplastics starts with the collection of starch material plants, which produce them by absorbing CO2 during photosynthesis. This plant starch is fermented by using lactobacillus bacteria, and is converted into a long-chain carbon polymer (PLA). These PLA granules are then molded into small plastic pellets which are melted to make different kinds of objects and packaging material. On the other hand, Polyhydroxylalkanoate is a polyester produced by fermenting raw vegetable materials such as carbohydrates, vegetable oil or even glycerine. bacterial strains. It is specially extracted from bacteria such as pseudomonas.

Bioplastics companies in India

The market for bioplastics in India is no longer nascent with many industry players taking pioneering steps. Our country has the raw material biomass required for bioplastics in abundance. Combining this with the rising awareness among consumers, India could become the potential fulcrum for global behavior change in turning away from plastics. Quite a few manufacturing firms like Envigreen, Ecolife, Plastobags, Earthsoul India and Truegreen have come up with different forms of bioplastics.

Truegreen is a firm based out of Ahmedabad that started a manufacturing plant with a capacity of producing 5,000 tons of bioplastics every year.

Plastobags is an established company that primarily started in the business of conventional plastics but recently has diversified its product portfolio and expanded into bioplastics with a whole range of products from carry bags, hygiene gloves to disposable waste bags and security bag.

Ecolife is a firm based out of Chennai that produces bioplastics for industrial packaging. Their products also include bioplastics for industrial packaging with different varieties like perforation films and lamination films.

Envigreen is the latest startup entering the Indian bioplastics market established by a Qatar-based NRI, Ashwath Hegde. In 2016, Envigreen opened its operations in Bengaluru and its production facility is already capable of producing 1,000 tons of bioplastics every year.

The growth of bioplastics in India is a positive change in consumer behavior and with continued support from the government and the citizens themselves, the awareness about bioplastics can become even more widespread. Hopefully more pioneering bioplastic companies like Envigreen, Truegreen and Ecolife expand the Indian market and bring about the much needed change toward a greener environment.

DEPARTMENT OF PHARMACEUTICAL REGULATORY AFFAIRS

INTELLECTUAL PROPERTY PROTECTION FOR ORPHAN DRUGS

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Abstract

Generally speaking, diseases that affect a small percentage of the world population are considered rare diseases or orphan diseases. The United States Congress defines a "rare disease or condition" as one that affects less than 200,000 people in the United States, or one that "affects more than 200,000 in the [United States] and for which there is no reasonable expectation that the cost of developing and making available in the [United States] a drug for such disease or condition will be recovered from sales in the [United States] of such drug." This definition explicitly acknowledges the economic dilemma associated with the huge investment required to develop treatment for a relatively small class of patients where, without sufficient exclusivity, the prospective return on investment may not justify the upfront research and development (R&D) costs.

Introduction

The definition of orphan and rare disease is different in different countries on the bases of the number of patients affected by them. In the United States of America (USA), according to the National Institutes of Health (NIH), 30 million Americans have one of the nearly 7,000 disease that are officially considered "rare" because every of these affect less than 200,000 people in the United States and affect greater than the 200,000 people, but for which recovery of cost of development and market is very challenging.

(1) The orphan drug designation depending upon the ratio of the number of patients affected by rare disease which is 7.5 per 10,000 individuals in the USA, 5 per 10,000 individuals in the EU, 4 per 10,000 individuals in Japan, 1 per 10,000 individuals in Australia etc. In the European Union at that time 5,000 to 8,000 different rare diseases affecting 6% to 8% population of it.

(2) Most rare diseases are genetic; because symptoms do not appear earlier they exist throughout the person's entire life. Some of the rare diseases can be occurring due to allergies or infections (bacterial or viral) or due to proliferative and degenerative causes for example the rare genetic disease Ribose-5- phosphate isomerase deficiency has been diagnosed in a single patient only. As the rare disease patient population is very small, rare diseases are generally neglected by the doctors and pharmaceutical companies. Conclusively, acts were made by different countries for regulation of orphan drugs and the introduction of a set of commercial incentives to try to stimulate the production of orphan drug products.

(3) The United States was the first country introduced an orphan drug act in 1983, after that number of other countries has followed the program, for example Japan (1993), Singapore (1997), Australia (1998) and the EU (2000). In Europe Union acts were made much later than the USA because it is group of 28 countries and its capabilities regarding the health is very much dispersed.

(4) The incentives given by governments to the developers and manufacturers of orphan drugs have led to growth of research in this field. A remarkable growth have seen in

orphan drug designations, 12% to 291 in USA and 62% to 201 in EU from 2013 to 2014. The orphan drug sales were increased 7.7% to $97 bn from 2013 to 2014 and it is estimated to grow by 11.7% per year from 2015 to 2020 to $178bn. The Orphan drugs sales will be become 20.2% of worldwide prescription sales of 2020 (excluding generics).

Today, there are over 5,000 known rare diseases, 80 percent of which have been identified as genetic in nature, with incidence of less than one in 2,000 people. Symptoms of some rare diseases may appear at birth, or develop later in childhood or even during adult life. Other rare diseases are the result of infections (bacterial or viral), allergies, or are caused by degenerative and proliferative conditions. Currently, the number of rare diseases for which no treatment is available is estimated to be between 4,000 and 5,000 worldwide.

Regulatory exclusivity and patent protection

Regulatory exclusivity and patent protection each provide critical incentives for drug development by providing appropriate mechanisms to keep generic competition off the market for a period of time sufficient to justify the expense and risk associated with drug development. The orphan context presents unique risks where the potential return on investment is inherently constrained by small patient populations and attendant reimbursement challenges.

Orphan Drug Act

Recognizing these challenges, certain countries have passed laws to provide additional incentives to encourage development of treatments for orphan diseases.

Orphan Drug Act in United States

In the United States, Congress passed the Orphan Drug Act in 1983 to foster the development and commercialization of drugs to treat rare diseases. The Orphan Drug Act includes a number of incentives designed to make the economic model associated with developing treatments for orphan indications more attractive. Most significantly, the Act provides for seven-year market exclusivity following the market approval of an orphan drug — in contrast, a traditional drug that is a new chemical entity (i.e., not previously approved for any indication) receives only five years of data exclusivity.

In addition, the Act provides for various tax credits, grants for drug development, fast-track approvals and expanded access to the Investigational New Drug Program. A number of other countries have enacted statutes designed to provide similar incentives.

Orphan Drug Act in Japan

In 1993, Japan introduced the Orphan Drug Amendment to the Pharmaceutical Affairs Law. It establishes three criteria for orphan designation:

1) The number of patients affected must be less than 50,000 within the Japanese territories;

2) There must be a medical need with no suitable alternatives, or the efficacy and safety of the drug to be designated must be better than available drugs or interventions; and

Orphan Drug Act in Europe

Europe enacted its Orphan Drug Regulation in 2000. The European statute designates as “orphan” a disease or disorder that affects fewer than five in 10,000 citizens. It establishes a period for market exclusivity of 10 years from authorization of an orphan drug product. A member state may not accept another application for marketing authorization or grant a marketing authorization for the same therapeutic indication for a similar medicinal product during that exclusivity period. The exclusivity, however, may be forfeited by the first applicant if the first applicant consents to a second application from another applicant; if the first applicant is unable to meet demand; if a similar product is found to be clinically superior; if, at the end of the first five years, a Member State shows that the product is sufficiently profitable not to justify maintaining its market exclusivity; and/or if the statutory criteria are otherwise no longer met.

Many other countries have introduced comparable legislation. For example, Singapore adopted orphan drug legislation in 1991, Australia in 1998, Taiwan in 2000, and South Korea in 2007.

Most recently, in October 2012, Canada announced that it will introduce a regulatory framework for authorization of orphan drugs. Jurisdictions with orphan drug regulations offer various incentives, including market exclusivity, tax credits, regulatory fee waivers and fast-track approval for orphan drugs. The particular incentives available vary from jurisdiction to jurisdiction.

Orphan exclusivity compared to data exclusivity

The most meaningful incentive provided by the various orphan drug statutes is the period of market exclusivity available for qualifying drugs. This to the first sponsor who obtains marketing
approval for a designated drug or biological product for the orphan indication. The US Orphan Drug Act of 1983 can be used as an example to illustrate how it works. The seven-year market exclusivity period provided by the Orphan Drug Act is granted only to the first sponsor who obtains marketing approval for a designated orphan drug. The exclusivity begins on the date that the drug receives Food and Drug Administration approval, and applies only to the orphan indication for which the drug has been designated and approved.

During the seven-year orphan exclusivity period, the FDA cannot approve an application using the same drug for the same orphan indication. It does not, however, preclude approval of either a drug using a different active moiety for the same indication, or the same drug for a different indication. The statute permits approval of a "clinically superior" product that uses the "same active moiety." In other words, if a competitor wishes to introduce a drug using the same active moiety for the same indication, the burden is on the competitor to prove that its drug is therapeutically superior when compared to the first drug approved for the same orphan indication. Thus, orphan exclusivity provides a meaningful period of protection from competition.

Orphan drug exclusivity

Orphan exclusivity differs from the data exclusivity provided by Hatch- Waxman Act and the more recently enacted biosimilar statute. The Hatch-Waxman Act provides five years of data exclusivity for new chemical entities (NCEs) not previously approved by the FDA. For biosimilars, an application for a biosimilar license may not be filed for four years after approval of the reference Biologics License Application ("BLA"), and its approval may not be made effective until 12 years after the BLA license. However, the data exclusivity provided by the Hatch-Waxman Act and the biosimilar statute merely prevents competitors from using an Abbreviated New Drug Application (ANDA) or biosimilar application, respectively, and does not prevent a competitor who runs its own clinical trials from marketing its competing drug product. In contrast, orphan exclusivity bars entry even from a competitor filing its own New Drug Application or BLA supported by its own clinical trials, so long as the competitor is seeking approval of the same drug for the same orphan indication, and other conditions are met (i.e., the competitor does not show clinical superiority). In this respect, orphan exclusivity is broader than data exclusivity. The scope of orphan exclusivity thus can be viewed in certain respects as analogous to the protection provided by a valid and infringed method of use patent covering the use of a particular drug substance (narrowly defined to exclude superior formulations) to treat the orphan indication, with a built-in injunction enforced by the FDA.

Orphan drug exclusivity does not extend to other uses for the same drug, but a valid and enforceable patent can. In this way, patent protection helps address the competitive risk associated with off-label use.

Orphan drug incentives at a glance					
	Market exclusivity	**Fast-track approval**	**Tax credits**	**Protocol assistance**	**Fee waivers**
Australia	5 years (as with other drugs)	Yes	No	Yes	Yes
European Union	10 years	Yes	Varies by EU member state	Yes	Yes
Japan	10 years	Yes	Yes	Yes	Yes
Singapore	None	Orphan drugs given priority in registration	No	No	No
South Korea	6 years	No	No	No	No
Taiwan	10 years	Yes	No	Yes	No
United States	7 years	Yes	Yes	Yes	Yes

The most important step in using patent protection to supplement orphan exclusivity is to patent the drug product composition of matter. However, because of the long development timelines associated with orphan development, maximizing exclusivity often will require additional filings covering advances made during the development process.

Conclusion

For orphan products with small and sometimes diffuse patient populations, the risk is particularly acute. The development costs are just as substantial, the development timeline often is longer, and the development risks associated with treating a smaller patient population often are greater. The somewhat longer (seven-year) and more robust regulatory protection alone often is not enough to justify the expense. A comprehensive patent strategy provides a key complement to orphan exclusivity by providing a buffer against the exceptions to orphan protection.

An IP strategy that is closely coordinated with clinical development is key to success. As explained above, companies should rigorously seek protection for novel compounds, formulations, manufacturing processes and any other innovations discovered as the development process progresses, to ensure maximum protection for the resulting orphan treatment and to supplement the exclusivity provided by orphan drug statutes. The best way to ensure sufficient return on the investment in orphan drug development is to combine the benefits of regulatory exclusivity and patent protection.

DEPARTMENT OF PHARMACOGNOSY

COMPARATIVE ANTIOXIDANT ACTIVITY OF PETROLEUM ETHER EXTRACT OF LEAVES, METHANOLIC EXTRACTS OF STEM AND ROOTS OF *OPERCULINA TURPETHUM*

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INTRODUCTION

The human being appears to be afflicted with more diseases from the early ages, taking advantages of plants growing around them to alleviate their sufferings from injury or disease with hopes for remedies in chronic diseases generated new enthusiasm in the research workers to develop herbal medicines.[1]

Herbal medicine most probably a major factor in the change of direction for health and the treatment of disease in the next century.

Primarily herbal medicines were used in the form of compresses and poultices or in the form of formulation like infusions and decoctions. However new analytical techniques like TLC, HPLC, GC, PC and pharmacological testing procedures, new plant drugs find their way into medicine as purified substances. The recent advancement in sophisticated instrumentation technology such as NMR spectroscopic and Mass spectroscopic techniques also helps to identify the chemical nature of the isolated plant constituents.

The effectiveness of medicinal plant lies in the varying complex chemical substances like alkaloids, glycosides, corticosteroids, essential oils, etc. which are the starting material for vast number of synthetic drugs.[2]

The natural plant products often serves as chemical models or templates for the design and total synthesis of therapeutically potent new molecules for the treatment of several diseases, for example, Taxol (*Taxusbaccata*), Ephedrine (*Ephedra gerardiana*), Vincristin and Vinblastin (*Catharanthusroseus)* and Allamandin (Allamandacathartica). The concept of drug design of some of the synthetic molecules has emerged out of the their quantitative structure activity relationship in terms of biodynamic constituents, for example, Belladona

alkaloids (Atropine), Quinine, Physostigmine, Cocaine, Morphine and Codeine have served as models for the design and synthesis of anticholinergics, antimalarials, anticholineesterases, benzocaine, procaine andother local anaesthetics. It has been estimated that over 5500 known plant alkaloidsand new ones are being discovered at the rate of one day.

Herbal drugs have provided themselves from ancient times. Tubocurarine, a muscle relaxant from *Chondrodendrontomentosum*, Diaboline, a antidiabetic substance from *Strychnospotatorum*, morphine, a strong painkiller from *Papaveraeasomniferum*, artemicin an antimalarial from *Artemisia* species illustrate the profound promise held by these drugs.

Natural plant based remedies are used for both acute and chronic health problems, from treating common cold to dreadful diseases viz., cancer, AIDS, diabetic, jaundice, schizophrenia, catastrophic cholera and terrible typhoid etc.[3]

The plant main constituents like alkaloids, glycosides, corticosteroids, flavonoids, essential oils, etc. are a part of the contribution of the herbal medicines and the use of the plants or their metabolites for therapeutic cure and treating an ailment is what we call the phytomedicine.

Ethnobotanical bioprospecting, which takes advantage of traditional medicinal knowledge and random 'grind and find' bioprospecting have been two methods of choice for phytochemical drug discovery.[4, 5, and 6.]

So, all these occurrences and facts encouraged the research over herbal medicine and therefore we can say the nature has come back for disease cure *Operculinaturpethum* is one among them.

Free radicals of different forms are constantly generated for specific metabolic requirements and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissue and bio molecules, eventually leading to disease condition, especially degenerative disease.

Some anti-oxidant activity plants[7]

S.NO.	PLANT NAME	FAMILY	PART USED
1	*Asparagus racemosus*	Lilliaceae	Root
2	*Andrographispaniculata*	Acanthaceae	Leaf
3	*Centellaasiatica*	Umbelliferae	Leaf
4	*Evolvulusalsinoides*	Convolvulaceae	Leaf
5	*Emblicaofficinalis*	Euphorbiaceae	Fruit
6	*Glycyrrhizaglabra*	Leguminosae	Root
7	*Terminaliachebula*	Combretaceae	Fruit
8	*Terminaliabellerica*	Combretaceae	Fruit
9	*Withaniasomnifera*	Solanaceae	Root
10	*Zingiberofficinale*	Zingiberaceae	Rhizome

METHODOLOGY

Collection of plant materials

Operculinaturpethum (L.)SilvaManso plant is a common weed throughout Andhra Pradesh. The *Operculinaturpethum (L.)SilvaManso* plant material is collect from local areas of **Vijayawada**, A.P.

Antioxidant activity[16, 17, 18, 19 and 20]

The term 'Antioxidant' refers to the activity of numerous vitamins, minerals and other phytochemicals to protect against the damage caused by Reactive Oxygen Species (ROS). By their ability to react with and damage many structures in the body, ROS are involved in various related physiological processes and diseases such as ageing, cancer and atherosclerosis.

Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants.

Free Radicals and other reactive oxygen species are formed constantly in human body and are removed by enzyme and non enzymic antioxidant defense system. The disturbance in redox homeostasis can damage lipids, proteins, carbohydrates, and DNA. Drugs with multiple mechanisms of **protective action including antioxidant properties may be always forward in minimizing tissue injury in human diseases**.

Free radicals of different forms are constantly generated for specific metabolic requirements and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissue and bio molecules, eventually leading to disease condition, especially degenerative disease.

Free radicals scavenging are the principal mechanism of the antioxidant function of the flavonoids, due to their capacity to donate electrons. Several flavonoids and other phenolic compounds are considered to be antioxidant not only because of their free radical scavenging activity but also because they chelate metals contributing to increased antioxidant capacity.

Various environmental, physical and chemical stresses on cells may induce either an overproduction of ROS or a deficiency of antioxidant enzymes. ROS are responsible for various cellular anomalies like protein damage, deactivation of enzymes, and alteration of DNA and lipid peroxidation which in turn leads to pathological condition like Carcinogenesis, Reperfusion injury, Rheumatoid arthritis, Diabetes etc. The regular intake of antioxidants seems to limit or prevent the dangerous effects caused by ROS. Thus, to maintain cellular health, it is important to have a specific and effective antioxidant that scavenges multiple types of free radicals so that it can be used in multiple diseases. Different *invitro* and *in vivo* test systems are available in the literature to assess the free radical scavenging activity of various compounds. Based on the efficiency of free radical scavenging, the compounds are classified in to strong, moderate and weak antioxidants.

In vitro methods are qualitative and they are used to find out whether the given compound is an antioxidant or not. However, IC_{50} values (concentration which can achieve 50% scavenging) or Trolox equivalents (free radical scavenging in terms of Trolox) can be used to quantify the activity. Also the methods are simple, colorimetric and there is no use of animal tissues.

Antioxidants are gaining a lot of importance as a panacea for a large number of life-style diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases etc.

Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.

Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects.

Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. That's why plants with antioxidant properties are becoming more and more popular all over the world.

So the above mentioned methods are used to evaluate the antioxidant activity of plants and drugs.

ANTIOXIDANT ACTIVITY METHODS

Reducing power method[21]

About2 mL of each sample and standard solutions were spiked with 2.5 mL of 1% Potassium ferricyanide solution. This mixture was kept at 50º C in water bath for 20 min. After cooling, 2.5 mL of 10% Trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min. About 2.5 ml of supernant was mixed with 2.5 ml of distilled water and 1 ml of 0.1% ferric chloride and kept for 10 min. Control was prepared in similar manner without samples. The absorbance of resulting solution was measured at 700 nm.

DPPH free radical scavenging activity

About 150 mL of DPPH solution was added to 3 mL methanol and absorbance was taken immediately at 516 nm for control reading. Different volume levels of test sample (20, 40, 60, 80 and 100 µl) were screened and made 100 µl of each dose level by dilution with methanol up to 3 mL. About 150 mL of DPPH solution was added to each test tube. Absorbance was measured at 516 nm in UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) after 15 min using methanol as a blank. The % reduction and IC_{50} were calculated as follows the free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

% antiradical activity = Control absorbance - Sample absorbance/ Control Absorbance X100

Each experiment was carried out in triplicate.

Nitric oxide free radical scavenging activity[22]

Sodium nitroprusside (10 mg) in phosphate buffer saline was mixed with different volume levels of test sample (10, 20, 30, 40, 50 and 100 µ L) made 100 µ L of each dose level by dilution with methanol. Incubate the solution at room temperature for 150 min. The same reaction mixture without the extract but equivalent amount of methanol served as control. After the incubation period 5 mL of Griess reagent was added. The absorbance was taken in UV-visible spectrophotometer at 546 nm. Ascorbic acid was used as positive control. The % reduction and IC_{50} were calculated as follows. Each experiment was carried out in triplicate.

Hydrogen Peroxide (H_2O_2) Scavenging Activity[23]

Hydrogen Peroxide scavenging activity of plant extract was determined using a modification of the method. About 4 mM solution of H_2O_2 was prepared in phosphate - buffered saline (PBS, pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorbance at 230 nm. Plant extract corresponding to 50, 100, 150, 200, 250 µ L of 1 mg/mL plant extract stock solution in 4 mL distilled water were added to 0.6 mL hydrogen peroxide in PBS solution. Absorbance of H_2O_2 was determined at 230 nm. Absorbance was determined 10 minutes later against a blank solution similar to that above.

% H_2O_2 Scavenging = Absorbance of control – Absorbance of test/ Absorbance of Control × 100.

CONCLUSION

The findings reported by the comparison of antioxidant activity of different extracts and different parts of (stems, leaves and roots) of OT.

The stem methanol extract is more anti-oxidant activity than other extracts. .

Further investigations are needed to identify the lead molecule and to elucidate the structure and exact mechanism of action for antioxidant activity.

EVALUATION OF ANTIMICROBIAL AND ANALGESIC ACTIVITY OF DIPLOCYCLOS PALMATUSFRUITS ON ALBINO MICE

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INTRODUCTION

The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases in their day-to-day practice. In traditional system of medicine, different parts of *Diplocyclos palmatus* were used. It is a short-lived, perennial climbing plant producing annual, much-branched stems up to 6 m long from a fleshy rootstock [1]. The stems scramble over the ground, climbing into the surrounding vegetation where it attaches itself by means of tendrils. It is more commonly known as the lollipop climber or striped cucumber (E), Shivalingi (H) belonging to family Cucurbitaceae. The plant is recorded in India as growing and spreading in the wild. When ripe, it is red with longitudinal white stripes and reminds one of lollipop, hence, the common name. Lollipop climber is a perennial climber with hairless stem, becoming thickened and white dotted on the ridges when older. Leaves are broadly ovate, 3.5–14 cm × 4–14.5 cm, lobes are linear shaped to elliptic, hairless. Leaf stalk 1.5–9.0 cm long. Flowers are small, white or yellowish, male in stalkless clusters of 2–8, along with five female flowers in the same axial. Sepal cup is 3–4 mm long in male, 1.5–2.5 mm long in female, sepals smaller than tube. Flower of male larger than female. Fruit is solitary. It is ovoid round, 1.5–2.5 cm. When ripe, it is red with longitudinal white stripes and reminds one of lollipop, hence, the common name. It is found including the Himalayas, at altitudes of 200–1500 m. The small flowers of Lingini or Shivalingi are of greenish-yellow in color. The female flowers of the plant are borne in fascicles and the male ones are solitary. The plant's corolla is about 3–4 mm, with ovate-oblong, acute, pubescent lobes. The fruit or berry of the plant is rounded, with a diameter of 2–3 cm and the bluish-green. They ripe red and bear a few brown, obovate seeds. The compressed seeds have a length of 4 mm and width of 3 mm, and they are usually encircled by a prominent raised band. The plant is generally flowers between the months of August and September and fruits in September and October in central India [2].

Phytochemical studies of D. palmatus show the presence of alkaloids, flavonoids, triterpenoid saponins, steroids and proteins, and resins with sugars and starch. The seeds have been reported to contain 12% oil, protein, along with goniothalamin, bryonin, punicic acid, and lipids. Disease and illness are very much related and having similar concepts. The concepts are mainly, patients suffer from "illnesses" and physician diagnoses and treats "diseases." Disease can refer to a combination of signs and symptoms. It can also be referred as a phenomenon associated with a disorder of function or structure or illness associated with a specific cause [3].

D.palmatus traditionally reported as an antimicrobial properties and analgesic properties, scientifically seed and leaves of plant already reported as an antimicrobial and analgesic activity. In the present study, fruits of D. palmatus are studied for phytochemical screening, antimicrobial, and analgesic activity. Seeds are used in sterility due to blocked tubes in

women, snake bite, root fever, stomach ache, and external abscess. Fruits are used in diarrhea; the Indian women sometimes take the seeds in combination with other plant drugs for helping conception and prevent miscarriage. The practitioners of ayurvedic medicine use the plant's fruit as an aphrodisiac and tonic, while in Siddha, the entire plant is used for getting relief from constipation. In vitro studies revealed that D. palmatus (aerial part) contain antioxidant and antimicrobial activity; seeds have analgesic,

MATERIALS AND METHODS

Plant materials

The fresh fruits of *D. palmatus* were collected from Moinabad rural area, Ranga Reddy district in the month of November 2016. Plant was identified and authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad, belonging to the family Cucurbitaceae.

Chemical and reagents

Chemicals reagents were purchased from S.D Fine and Merck Chemicals (Mumbai, India). Albino mice were purchased from Sanzyme animal house, Gaganpahad, Hyderabad, Telangana – 500 077. Animal studies were performed in MAK College of Pharmacy, Moinabad. The organisms *Escherichia coli* (Gram-negative) bacteria and *Bacillus subtilis* (Gram- positive) were obtained from soil of MAK College of Pharmacy by isolation technique. Aspirin, streptomycin, and ciprofloxacin were purchased from local medical shop.

Instrumentation

Autoclave, Soxhlet apparatus, Petri dish, heating mantle, incubator, mice cage, hot air oven, laminar air flow, analgesiometer, water bath glass, etc., were obtained from MAK College of Pharmacy.

Preparation of extract

The fruits of the plant are shade, dried, and powdered. The extraction of powdered fruits was performed by hot extraction method by Soxhlet apparatus using ethanol. The extract was then filtered respectively through Whatman filter paper to remove impurities and volume is reduced by vacuum evaporator and then dried, collected, and finally stored in well-closed container at room temperature until it is used for further studies in this experiment [8-10].

Test microorganism

The test microorganisms were clinical isolates from the stock culture of MAK College of Pharmacy, Moinabad, Ranga Reddy. They include *E. coli* (MTCC 1698) and *B. subtilis* (MTCC 2757). Each of these microorganisms was subcultured into nutrient broth to test for viability and subsequently on nutrient agar slants and kept at 4°C before susceptibility testing.

Animals

To study the analgesic and anti-inflammatory activity of the extract, male and female albino mice were used in the present study. In the present study, male and female albino mice were used. The animals were maintained in clean polypropylene cages with 12 h light and dark cycle at a temperature of 26–28°C and supplied with pellet diet and water *ad libitum*. The

animals were acclimatized to laboratory conditions for 1 week before starting the experiment. The experimental protocol approved by the Institutional Animal Ethical Committee allotted registration No. 1970/PO/RE/S/17/CPCSEA.

Phytochemical screening

The phytochemical investigation of ethanolic and aqueous extracts of *D. palmatus* fruits was carried out by various standard procedures for the detection of secondary metabolite such as of alkaloids, tannins, flavonoids, and triterpenoids. Thin-layer chromatography (TLC) was also performed for bryonin and punicic acid (identified as active components) using mobile phase of acetone: toluene:ethyl acetate (1:1:1) [10,11].

Table : Antimicrobial activity of ethanol extract of *Diplocyclos palmatus* fruit (1 g/ml)

Name of extract	Microorganism (zone of inhibition in mm)	
	Gram-positive *Bacillus*	Gram-negative *Escherichia coli*
Ethanol control	12	19
Ethanol extract of streptomycin (1:1)	8	13
Ethanol extract of ciprofloxacin (1:1)	11	9
Ethanol extract of streptomycin (1:2)	10	15
Ethanol extract of ciprofloxacin (1:2)	9	7
Ethanol pure extract control	8	15
Ethanol pure extract (1:1)	12	14
Ethanol pure extract (1:2)	11	13

Fig. 1: Thin-layer chromatography of *Diplocyclos palmatus* fruit extracts (acetone: toluene:ethyl acetate) (1:1:1)

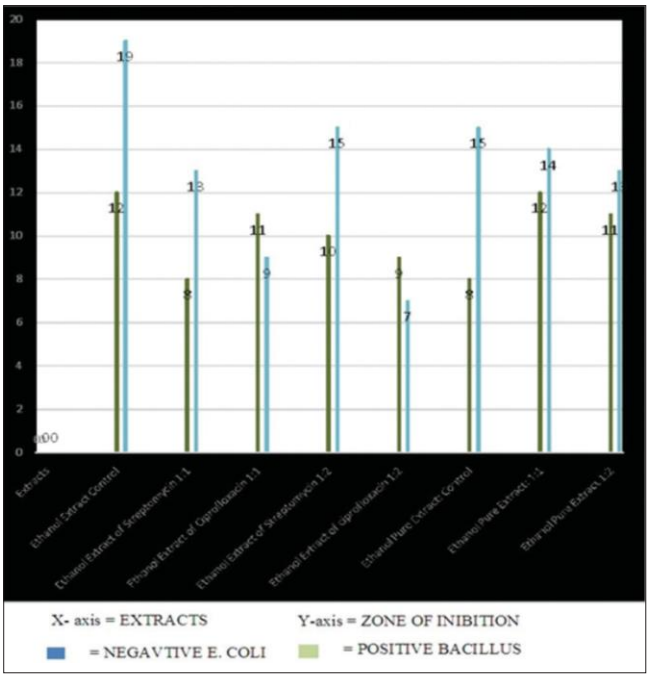

Fig. 2: Graphical representation of antimicrobial activity

DISCUSSION

To investigate the medicinal value of plants is an ancient concept and for centuries people have been trying to develop various drugs from plants which have potent activity and less side effects. In literature, it has been indicated that medicinal plants are the foundation of conservative medicine and the activity of plant extract is due to different chemical agent in the extract which was classified as active compounds [18]. In plants, various phytochemical constituents such as tannins, flavonoids, alkaloids, and several other aromatic compounds are available and secondary metabolites of plants that serve as safeguarding mechanisms against predation by many microorganisms, insects, and herbivores and act as a phytoprotectants and respond to environmental stress condition [19]. This may, therefore, explain the demonstration of antimicrobial activity by the fruit extracts of D. palmatus. It has been also reported that D. palmatus fruit extract shows antimicrobial property due to the presence of secondary metabolites such as phenols and flavonoids.

The demonstration of antibacterial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [20]. Results of this study demonstrated that the Gram-negative bacteria (E. coli) were more resistant than Gram- positive bacteria (B. subtilis). It was observed that the plants in different regions of the world had antimicrobial and analgesic effect. This may be due to many factors such as the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity, and composition of extracted product, and different bacterial strains [21]. D. palmatus fruits are more significant when compared with standard drug ciprofloxacin and streptomycin [2]. Due to presence of lipopolysaccharide layer in outer membrane of Gram-negative bacteria, its hydrophobicity acts as a strong permeability barrier against hydrophobic molecules [22]. Hydrophobic molecules can pass through cell wall of Gram-positive bacteria easier than the Gram-negative bacteria because the cell wall of the Gram-positive bacteria contained only peptidoglycan [23]. Ethanolic extract fruits of D. palmatus more significant while compare to the other parts of D. palmatus in antimicrobial activity [24].

The analgesic properties of the fruit of D. palmatus were studied using three laboratory models, which allowed the assessment of responses to chemically induced pain stimulus (aspirin). The radiant heat method is the most reliable test for analgesic. The analgesic activity of the ethanolic extract of D. palmatus fruit produced significant graded dose effects in three models employed, namely tail clip, tail immersion, and radiant heat method, in compare to the other parts of
D. palmatus [25].

CONCLUSION

The antimicrobial activity of the ethanolic extracts of D. palmatus fruits against the test organisms showed significant antimicrobial activity as proven by testing against the microorganisms that are B. subtilis (Gram- positive bacteria) and E. coli (Gram-negative bacteria). Analgesic activity on different groups of albino mice suggested that the plant also possess significant analgesic activity. The analgesic and antimicrobial activity of the ethanolic extract of D. palmatus fruits are more significant compared to the other parts of D. palmatus. Hence, the study concludes that plants have both analgesic and antimicrobial activity and, therefore, with it can be used for various therapeutic purposes and further analysis.

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- Adopt and implement best practices for learning and research.
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