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B.PHARM-PROGRAMME VISION & MISSION

Vision

To excel in pharmaceutical education and research and provide outstanding professional to the health care need of the society.

Mission

To educate students by providing a vibrant environment and inculcate critical thinking, problem solving, leadership skills, ethical values and life-long learning. The institute aims to be an enabler to prepare students to meet the changing environment of the health care industry by developing competent professionals.

B.PHARM-PROGRAMME EDUCATIONAL OBJECTIVES

PEO 1: To acquire effective knowledge of pharmaceutical sciences to be able to lead the industry and health care sector

PEO 2: To attain practical training and technical expertise in pharmaceutical sciences

PEO 3: To inculcate professional and ethical standards with effective interpersonal communication skills.

PEO 4: To develop interdisciplinary & holistic approach in Pharmaceuticals for societal cause, problem solving and lifelong learning.

PEO 5: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking.

B.PHARM-PROGRAMME OUTCOMES

No.	Programme Outcomes
PO1	**Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
PO2	**Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines
PO3	**Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.

PO4 **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations

PO5 **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.

PO6 **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).

PO7 **Pharmaceutical Ethics:** Honor personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.

PO8 **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions

PO9 **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice

PO10 **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

PO11 **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis

PO12 **Drugs and diseases:** Understand different classes of drugs, their mechanism of action, dynamics, kinetics, structure activity relationships, pathophysiology and pharmacotherapeutics of various diseases.

PO13 **Drug development:** Ability to synthesize, develop and/or evaluate various pharmaceuticals and their formulations and cosmeceuticals products

PO14 **Analytical skills:** Develop skills in qualitative and quantitative analysis of various pharmaceuticals.

PO15 **Training:** Acquire technical knowledge and hands on training on equipments, instruments and software used in the field of pharmaceutical sciences.

**- PO12-15 are program specific outcomes*

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Content

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EDITORIAL MESSAGE

India's pharmaceuticals market has grown at the rate of 13 to 14 per cent in confidence and firmly moved on to an accelerated growth path in last five years. Also there is a growth in medical infrastructure and health insurance coverage. The treatment of chronic diseases has gone up. The remarkable success of a few recent launches has demonstrated the true potential of patented products. Backed by solid fundamentals, the market is giving rise to a variety of business opportunities.

Globally, the U.S. Food and Drug Administration approved Daurismo (glasdegib) tablets to be used in combination with low-dose cytarabine (LDAC), for the treatment of newly-diagnosed acute myeloid leukemia (AML) in adults who are 75 years of age or older or who have other chronic health conditions or diseases (comorbidities) that may preclude the use of intensive chemotherapy. At national level, the drug price regulator National Pharmaceutical Pricing Authority (NPPA) fixed prices as well as revised ceiling and retail prices of 68 formulations, including those used for treatment of diabetes, blood pressure and HIV.

It is pertinent to be updated with the current scenario in the pharmaceutical field. In this issue we have invited articles from renowned experts together with postgraduate and undergraduate students spread across different facets of pharmaceutical field.

We are thankful to all the faculty members, HODs, our Director as well as our Director General Dr. Anup Singh and the Nirma University authorities, stakeholders, reviewers for all the support provided in rolling out this issue. We hope that our efforts will help the readers to widen the horizons.

We look forward to your constructive criticism and suggestions.

Happy reading!!

Editorial Team, NUJPS

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REVIEW ARTICLE

SOCIO-ECONOMIC IMPACT OF CLINICAL RESEARCH IN VARIOUS COUNTRIES

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Abstract:

This article attempts to outline the socio-economic impact of clinical research in sample geographies from around the world. With an attempt, we could sample geographies that largely represent varied population - from developed markets to developing markets, from poor to rich countries, from countries seeing increase in clinical research to countries that are struggling to retain clinical research. In absence of any research/ survey that can map socio-economic impact at global level, this article best describes the general trends at least, if not a truly global view. Largely, there are two impacts which are quite visible across all of the sampled geographies. The first one is that the advent of clinical research in any geographies have led to a palpable increase in the standards of medical care in that geographies. This is probably driven by essential training that researchers go through and thereby learning the GCP - Good clinical practice. Another effect is a definite increase in the standards of medical infrastructure, once the clinical research started. This is a reflection of the fact that general population has benefited by investment made by sponsor companies to perform clinical research. The second impact is that the effect of Clinical research activities on economy is also uniformly positive. This results from new job creation that leads to downstream economy. But additionally, it also follows from having healthier humans (those who received medical benefits of clinical trials) who pay more taxes and who further pedals the economy by spending. All in all, the size of these benefits (as measured in various markets) is significant enough not to loose on them and many countries are actively pursuing clinical research to get these benefits.

Keywords: Clinical Research, GCP, Clinical trials, economy

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Introduction:

"Research is formalized curiosity. It is poking and prying with a purpose" -Zora Neale Hurston, An influential author of African-American literature and an Anthropologist.

As Zora Hurston put down, Research is essentially poking and prying in a very crude sense. Accidental "poking" over data of daily occurrences (and subsequently, much more intrusive efforts); and "prying" on the patterns of those occurrences - led humans to build knowledge; that ended up discovering world's oldest and most widely used group of anti-bacterial agents called Penicillins. Many such discoveries of yesteryears were accidental; but they became the foundation of scientific approaches to biopharma R&D. We are benefitting from these approach even today and keep maturing them further. The act of Drug development has since evolved significantly and has been more or less standardized, across the globe.

Current drug development pathway is largely defined by requirements or specifications that Regulatory agencies worldwide have built in their respective regulations. However, they are a reflection of innumerable scientific, cultural, economic, social and other factors (or incidences e.g. Thalidomide tragedy) that influenced the pathways design/ specifications. A case in example is changes in FDA regulations that resulted from the knowledge about potential of certain classes of drugs for QT prolongation. FDA provided a detailed guidance for Pharma industry for this element and a new business, 24/7 cardiac monitoring during clinical trials came into existence. New information/ knowledge about possible risks - affect how FDA and other regulatory authorities assess (and hence influence) drug development efforts. It is a classical chain reaction - where knowledge from today's research influence the research pathways of tomorrow's dugs and that results into further downstream effects like more expenditure, longer wait for drug, higher barriers for research, more patients in trials - before a drug is born.

Between 1928 (when Penicillin was first discovered) and 1942 (when it started being widely used to treat infections[1]) - many patients had to die, waiting for a cure that Penicillin could have offered. While the wait of 14 years was a result of an accidental journey that Penicillin had to go through - all of our newer biopharmaceuticals (NCEs and NBEs) mandatorily need to go through a similar journey called Clinical trials, before being approved for use in market conditions.

Since clinical research is arguably the phase of drug development that consumes largest amount of (a) R&D investment (b) R&D time and (c) R&D human efforts - it is bound to have the largest impact on humanity - socially and economically.

This article attempts to review such socio-economic impacts of clinical research. As with any other socio-economic analysis, we have had to put many "informed"

estimates in quantifying the impact. Additionally, we had to build this article as compilation of similar, smaller articles, separately written for various geographies, rather than a holistic worldview (which would have been interesting, enormous - but erroneous), for want of standard data-sets from all countries.

Clinical research:

Clinical research may loosely be defined as any stage of research where an un-approved research drug (to include device also) is introduced in humans. This does not include accidental or un-intentional exposure, neither has it referred to any activities that's not in alignment with ethical principles mentioned in the Declaration of Helsinki - first introduced in Jun 1964 and subsequent updates.

Because of the risks associated with such experimentation - methodology of Clinical research has been designed to be quite staggered, where each new phase of development follows (a) a careful review of the data produced in the previous experiments (b) and the fact that the latest data justifies moving ahead to the next phase of research. Essentially this leads to a "Linear" path of drug development and hence not always the most efficient way of development. However, Regulatory agencies in collaboration with biopharma industry worldwide, have come up with more efficient and scientific approaches for clinical research (e.g. Adaptive clinical trials, experimental IND etc), without increasing the risk.

The process of clinical development is broadly classified as below:

Phase 1: This phase of clinical research involves exposure of the study drug to a very small number of people (mostly healthy volunteers, but patients in some settings). The objectives associated with this phase of clinical research are largely driven by "safety needs" rather than "efficiency". However, studies in this phase may also reveal many other elements related with PK and PD behavior of the research molecule e.g. Absorption profile in humans, Interactions with food or other influences, Bioavailability of the molecules and information about metabolism and excretion. Some literature further classifies this phase in Phase 0 and then Phase 1 - which is a semantic differentiation. In these literatures, Phase 0 clinical research is defined as micro-dosing or sub-therapeutic dosing of the Investigational drug to achieve the same objectives (PK,PD& sometimes cellular level information) mentioned earlier. There are no set guidelines on number of human volunteers/ Patients that can be subjected to this phase (0 or 1) - but literature puts this number as anywhere between 20 to 100.

Phase 2: In this phase of Clinical research, the development objectives are expanded to get more information on how the Investigational drug behaves in patients (as compared to healthy volunteers in Phase 1), what's the safety profile of the drug in patients, what's the efficacy behavior of

the drug in patient (early indications), How optimum is the formulation and whether any formulation changes are needed etc. Phase 2 essentially determines whether the Investigational drug can be tested in larger number of patients or not. Depending on the variety of objectives for the research - there can be multiple Phase 2 trials running in parallel or overlap or sequence. There are no set guidelines on number of patients required in Phase 2 - but usual numbers are upto 300 patients. Phase 2 trials can also be sub-classified as Phase 2a and Phase 2b - where Phase 2a indicates a focus on safety while Phase 2b indicates focus on efficacy in patients.

Phase 3: This phase indicates the large scale exposure of the Investigational drug to patients with targeted clinical profile. By the time a drug reaches Phase 3 trials, a significant data is already available for the safety of the molecule in patients and focus is to ascertain efficacy objectives and safety profile of investigational drug in larger patient population. There can be multiple Phase 3 trials running in parallel and/or sequence, where one of them serves as "Pivotal" trial. The results from this Pivotal trial forms the basis of marketing approval from the regulatory agencies. Other trials in this case serves to provide supplementary evidences, dosage info, refined target patient population etc. This phase (phase 3) can also be further sub-classified as Phase 3a - that denotes continued focus on safety and 3b that denotes focus on efficacy objectives. There are no set guidelines for number of patients involved here too - but general size varies from 300 to 3000 patients depending on the statistical model. **At times, these trials are also used to collect other ancillary data for evaluation of economic benefits, comparison of patient experience amongst competing therapies.**

Phase 4: This phase of clinical research is a connecting link between clinical research that's done in highly regulated research setting and actual exposure of the approved product to the masses. This phase of research may be indicated as necessary by regulatory agencies while according marketing approval OR may be done by the sponsor company to gather additional information that helps in marketing message for the drug. Largely, these trials are aimed at collecting data of wider exposure of the drug, including HEOR (Health economics & Outcome research), Post-marketing obligations to submit AE data etc.

Why study impact of clinical research on humanity?

As mentioned above, out of all R&D activities - Clinical research can have the biggest impact on all of us, because these research efforts are highly intertwined with how we live (also in alignment with the objectives of clinical research i.e. exposing Investigational drugs in controlled, scientific and ethical way to human beings).

A classical example[2] of how clinical research affects the society - is probably as old as first attempt of conducting clinical research on humans.

The first recorded experiment resembling a clinical trial was not conducted by a medical, but by King Nebuchadnezzar a resourceful military leader. During his rule in Babylon, Nebuchadnezzar ordered his people to eat only meat and drink only wine, a diet he believed would keep them in sound physical condition. But several young men of royal blood, who preferred to eat vegetables, objected. The king allowed these rebels to follow a diet of legumes and water — but only for 10 days. When Nebuchadnezzar's experiment ended, the vegetarians appeared better nourished than the meat-eaters, so the king permitted the legume lovers to continue their diet. It is hard to imagine now - but classically demonstrates how human beings may be impacted by conduct of clinical research.

In current times, Clinical research is very intensely governed by regulations based on ethical principles (which obviously have very high human touch) and its impact on our life has only increased since 562 BC (the time of Nebuchadnezzar).

Having established the point that there is a materially significant impact of Clinical research activities on human society, let us explore some general phenomenon that reflect the impact of clinical research on the world:

Globalization of clinical research:

While the earlier Pundits of Clinical research & the Regulatory agencies defined the phases of Clinical research - they would not have an idea how far this research will grow. This is evident from the fact that none of the definitions of Phases i.e. Phase 1, 2, 3 or 4 - define trials in terms of geographic spread, ethnicity of the participants etc. It just speaks of the research objectives & number of volunteers/ Patients. Global spread of clinical trial is dealt with, in a case by case manner within a wide spectrum in a pivotal trial (all patients from USA to no patients from USA).

As evident from the infographic[3] below - Number of clinical trials intended for submission to the US-FDA have proliferated far, wide & thick. The 4 snapshots below captures the number of trials registered on clinicaltrials.gov in 4 decades, from 1980 to 2018. It paints a very interesting picture of how clinical trials have spread over the years.

Driven by the economics of the effort - globalization of clinical research has led to variety of effects on us, some of which we will review in country specific analysis also. However, the most important effects of globalization of clinical trials are:

- Increase in number of uniformly trained (ICH-GCP) Investigators & clinical research professionals around the world
- Access to newer medicines/ devices/ procedures - in the remotest regions of the world
- Downstream economy from activities that support clinical research in newer region

Impact of process of Clinical research on health service outcomes[4]:

While the Clinical research activities proliferated across the world - global healthcare markets did not necessarily have similar service standards. Worse, even within same countries; healthcare service levels varied significantly from each other, based on factors like Government facility v/s Private facility, Self-pay versus Payers, Rural v/s Urban and more. Clinical research got globalized on top of vastly dissimilar healthcare standards in different markets.

Did the globalization help in equalizing the healthcare standards?

While there is ample literature that shows "first translation gap" i.e. the gap between Laboratory research and Clinical research, there is hardly any literature that described "second translation gap" i.e. difference between Clinical research (conducted in dissimilar settings) & how the results from these trials are implemented in real-world. A workshop was organized in September 2009 by International Agency for Research in Cancer, at Lyon, France - that focused on dwelling upon this aspect. The participants in the workshop came out with some startling view on how the research activities affect the service standards in healthcare facilities:

- Participants strongly agreed on importance of the subject - Comparing the Healthcare service outcomes between trial participants and non-participants in similar set-up. However they also agreed that it was quite difficult to study this for variety of reasons.
- There was preliminary evidence to suggest that a Research active system improved clinical performance.
- There have been efforts all around the world, to develop comprehensive infrastructure within healthcare system to support and promote clinical research.

While the workshop could not establish evidence or direct link between clinical research and improvement of Health service outcomes, there are enough of surrogate evidences to suggest that service standards amongst erstwhile heterogeneous countries - are increasingly being "assimilated" because of Clinical research.

Social Media and Clinical Research:

Because of the ethical & confidentiality regulations around Clinical research, one might expect not to see much information/ engagement of trial subjects through social media. However, Social media having become such an inseparable part of our "being" nowadays - early signs of a huge impact (that social media can have in future) are becoming increasingly visible[5]. Some examples:

- Many of the frontrunner regulatory agencies e.g. US-FDA and EMA - have come out with guidance documents or other communications,

attempting to address the usage of social media. At this stage, the guidelines are quite general - but they are certainly a welcome sign.

- Novartis used a Twitter feed to boost awareness about a phase 2 trial involving stomatitis and breast cancer, and others have used textmessaging.

- Many regulatory agencies as well as Pharma companies, routinely monitor social media feeds to identify and process Adverse events, reported by patients in social media feeds (social media listening).

- Companies routinely build specific websites for their large clinical trial programs. Currently, the scope of most of these website stays limited to engaging investigators, CRO teams and others. However, with clearer guidelines, Social Media can become the choicest of tools, to achieve Patient centricity in clinical research.

Millennial and their influence on Clinical research:

While we are dwelling upon how Clinical research has impacted humanity, it might also be a very interesting to see how the new generation of millennial physicians is changing clinical research itself. In some ways, the active engagement of millennial generation to change/ challenge the process of clinical research itself indicate a socio-economic impact of clinical research! Few very interesting examples are given below:

- Early & intense collaboration: Matthew Howes, executive vice president, Strategy & Growth for PALIO, wrote, "We should expect this generation to tear down walls between sponsors, vendors, and sites involved in clinical programs. Drug development of the future will see research sites and investigators brought in, before protocols are developed to create a highly collaborative team environment". This prophecy statement written in 2011 - looks quite real now in 2018 - just over past 7 years.

- Technology integration: The advent of technology in clinical research has been much fast paced in recent years, compared to the first 3 decades of clinical research. This is a reflection of millennials' willingness to think beyond the legacy inefficient systems and integrating newer technologies in research activities. Online tools like RateClinicalTrials.co.UK, PatientsLikeMe and Yelp - significantly engage patients and improvise their participation. Movement of trial data from disjointed databases to integrated clouds - is making the decision process quick and efficient for Sponsors.

While we did highlight the positive side of impact of clinical research, we must also not forget about following general issues associated with clinical research:

- **Ethical issues**: The issues around problems in Informed consents, how "voluntary" the consent is etc.; are frequently referred to in global media. While these might not be 100 % substantiated, it is quite essential that all we form our regulations and practices in a way that can ensure an over-compliance to the ethical principles in Declaration of Helsinki.

- **Access to trial V/s Access to medicine**: While clinical trials give an early access to the newer medicines in a research setting, it does not guarantee the access to successful products as companies don't always launch it (soon enough) in the market that they use for trials.

We will now study country specific impact of clinical research as they are studied and reported:

Africa, Burkina Faso: Africa has generally witnessed a lot of research activities for diseases like HIV, Infectious diseases and especially Malaria. Largely these activities were sponsored by philanthropic organizations as well has world health bodies like WHO, UNICEF and many others. The research proliferation has been heterogeneous with some of the geographies having received a lot, while some have not seen any research, despite having a lot of disease burden.

Conducting clinical research in African continent is quite challenging because of infrastructural as well as geo-political issues. However a sample of how clinical research can positively benefit the general population, has been nicely documented in a case study[7] reported from Burkina Faso.

The need for conducting clinical trial in Malaria in the region, led to creation of Clinical Research Unit of Nanoro (CRUN) - which effectively led to building of an entire ecosystem to conduct International standard clinical research. Following tangible changes were achieved:

- Between 2008 and 2013, a fully functional, ICH-GCP compliant research facility was established in Burkina Faso that attracted a total of 25 research grants from Private and Government agencies to conduct more research.

- Research team grew in the same time from 10 to 254.

- A Health and Demographic Surveillance System (HDSS) was set up, that covered a total population of about 60,000 people spread in 24 villages.

- The research facility got the electricity connection from National grid, which was then extended to entire village, resulting into positive engagement of population.

- A clinical laboratory was set up (first in that region) with modern equipment, resulting into a positive outcome in overall healthcare provision scenario.

While in rural, undeveloped geography clinical research may generate negative perceptions; socially engaging efforts like the above can build a positive environment for people and en ecosystem to conduct more research. Efforts like these, can also contribute to the micro-economy of poorest of the poor regions.

Australia:

Despite its very thin population density, Australia has very proactively aligned its healthcare system to attract global Clinical research. The impact of the trial activities is very clearly evident in two separate reports [8,9] published in 2017.

The first report[8] attempted to estimate the value generated by Clinical research, by measuring Economic activity. This study considered the clinical research activities including both Industry sponsored Clinical trials as well as Investigators Initiated trials. Following infographic suggests a holistic representation of value generated by clinical research, directly as well as through downstream effects:

The findings (below) from this study are suggestive of huge impact on Australian healthcare system (and hence on population, in general):

- A total of 1360 trials started in 2015 and there were 6,900 trained professionals were available to support these trials.
- Total direct expenditure for ongoing trials in 2015 - was estimated at $1.1 billion (for a comparison, entire Australia's expenditure on Health and Medical R&D was about $4.3 billion in 2008).
- The above expenditure leads to downstream (flow-on) benefits to the participating patients as well as to the sector. This led to a multiplier impact, by having more spending by those earning from these activities, as well as more healthy individuals who paid taxes to the economy.

The anticipated economic benefits from R&D investment are perceived so "Assured/ Guaranteed" that Australian government has created a designated fund - MRFF for the same. MRFF will receive any savings from Health and Hospitals fund (HHF) and it has already grown to the tune of $4.6 billion in 2016. This MRFF will fund Investigators Initiated Trials as well as initiatives that can grow Australia as a world class Clinical trial destination.

Another study[9] from Australia attempted to evaluate the economic impact of Investigator Initiated Trials (subset of all trials), on a sample set of 25 trials and extrapolated the results from that detailed assessment to derive the below conclusions:

- Gross benefit for the 2014 year was estimated at $ 2 billion resulting from better health outcomes and reduced healthcare service costs.
- Reduction in healthcare service cost (on account of clinical trial activities)

was about 30 % of the gross benefit $ 580 million and it was larger than the total cost of three Trial Networks from 2004 to 2014.

- The overall consolidated benefit-to-cost ratio for the networks is 5.8:1, or a return of $5.80 for every $1 invested.
- The results of the 25 trials only needed to be implemented in 11% of the eligible patient populations for benefits to exceed costs.
- For every $1 awarded in National Health and Medical Research Council (NHMRC) grants to the 25 trials, a return of $51.10 was achieved.
- Just 9% of the $2 billion gross benefit from the trials in this study, was equivalent to all NHMRC funding received by all Australian networks between 2004 and 2014.

However startling the above numbers may look like, they surely point to a conclusion that Clinical research conducted in a country, results in a positive pay-back to the healthcare system and Australian government is proactively leading to gain these benefits.

Belgium:

Belgium historically holds a leadership position in participating in Clinical trials with a per-capita participation in trials holding as high as 9%. Belgium is also one of the world's leading countries in terms of site density i.e. number of sites per a million population. Belgium's position is only second to the USA.

However the landscape of Clinical research is changing and Belgium is fast losing its position as a preferred destination for clinical trials, to emerging countries.

Stakeholders from Belgium's pharma industry, regulatory bodies and healthcare network engaged reputed consultants PwC to research the current position of Belgium (in 2012) as well as to prescribe initiatives that can increase the clinical research activities and hence benefit from them[10]. Following data from the report - highlight the impact of Clinical trial activities on Belgian population:

- Clinical trial generate employment and contribute significantly to the local economy and also help translate the knowledge in better ways to treat diseases and improve healthcare.
- Although Belgium represents only 2.7 % of European GDP, its pharmaceutical industry represents a higher share of employment at 4.9% as well as higher R&D investment (6.6%) within Europe. This is reflected in the fact that percentage of people employed in R&D (4,600) out of total number of people employed by Belgian pharmaceutical industry (i.e. 32,200) is high at 12 %.
- Clinical trials constitute a significant share of these higher employment and investment. As surveyed in select

hospitals, 13 % of their annual budget was coming from income generated from Industry sponsored clinical trials.

Brazil[11]:

Brazil also has been having its fair share of clinical trials over past 20 years. Average relative rate of growth of trials (listed on clinicaltrials.gov) in Brazil is at 16 % versus the general growth experience outside Americas at 15%. However there is a significant need & capacity to conduct more clinical trials.

This is because Brazil enacted in 1990 a Unified Health System, which decentralized the provision of healthcare at Municipalities level. This healthcare is provided free of cost to the patients in need and who are not having any means. However the recession and local political situation has led to a decrease in the budget availability for the Universal Health System by $ 1.1 billion. So while the burden of diseases like Cancer is rapidly increasing in Brazil, available budget for the same is decreasing.

Brazil is consciously putting efforts in place that will facilitate more clinical research in Brazil and thereby (a) help Brazil's healthcare system in attaining global standards and (b) reduce the economic burden by having more patients receiving medical care by clinical trials. Below are some recent initiatives:

- A new law is under approval mechanism to expedite the regulatory approvals (which currently takes nearly 1 year). Even before it is enacted, the agencies have started reviewing the trial applications in 6 months time and hence this is already being achieved.
- On lines of similar efforts in other LatAm countries, Brazil is also coming up with Cooperative groups for Cancers. This will have a huge impetus on bringing patient awareness, getting better epidemiological data recorded and hence making it more transparent for sponsor companies.

Canada:

Canada has embarked on a mass collaboration to help her regain the human, social, and economic benefits of clinical trials[12]. There is a clear understanding and vision amongst all stakeholder groups about the economic and healthcare benefits that the country can gain by conducting Clinical research. Unfortunately, Canada is losing clinical trial opportunities that allow patients access to leading-edge drugs/devices, keep researchers at the forefront of clinical innovation, and generate economic benefits. While Canada offers great science, comparator countries who are more nimble at initiating trials are becoming preferred partners for industry investments.

To rebuild Canada's advantage, industry, academic healthcare, government and others have agreed on an action plan; secured resources and political will; and

begun initial work. To expedite progress, a Canadian Clinical Trials Coordinating Centre (CCTCC) is set up and being funded by Canada's Research Based Pharmaceutical Companies (R&D), the Canadian Institutes of Health Research (CIHR), and the Association of Canadian Academic Healthcare Organizations (ACAHO). Also, all stakeholders got together and built a nine point consensus plan, to attract more clinical trials in Canada. Following actions are being planned:

- Building of a National Advisory Panel - one single body to lead and coordinate measures to increase clinical research activities in Canada.
- Under the leadership of CCTCC, following trust building measures would be included in the action plan:
- Shared goals for all stakeholders and commitment for shared actions too
- Capacity (training of resources, building of coordinating centres, awareness amongst the patient population) building efforts, ultimately leading to more trials in Canada and more participating patients

As is evident from the above directional goals/ action items - will have an immense socio-economic impact on population.

Denmark:

Denmark has a population of about 5.3 million and had a GDP of USD 306 billion in 2017. For a relatively smaller country and economy like Denmark, it has significantly accurate estimates of socio-economic impact of clinical trials in the country. Below statistics is derived from an executive summary of "The value of Clinical trials in Denmark" that was published by Copenhagen Economics in July 2017. It lists some interesting facts as below:

- Each clinical trial initiated by the industry improved Danish GDP by an average of 902,000 kroner & boosted public finances by an average of 1,169,000 kroner.
- For every 1 krone spent by private companies on clinical trials in Denmark, a 64 øre increase in GDP is generated. This return is in far excess compared to the money invested by the pharma companies and indicates that placement of trial in the country is important.
- In 2015, Pharmaceutical companies spent 248 million kroner in clinical trials, thereby improving the quality and capacity of the public health sector. In practice, this expenditure occurred by paying for the time of doctors and nurses, as well as by sponsoring medicine and medical equipment employed at hospitals.
- On an average, 1 clinical trial initiated by the industry generates 5.3 FTEs employment, consisting of 3.1 FTEs in the private sector and 2.2 FTEs in the public sector.

- For every 1 million kroner a pharmaceutical companies invested in clinical trials, employment of 1.3 FTEs is generated, consisting of 0.5 FTEs in the public sector and 0.8 FTEs in the private sector.

Hungary:

Statistics reported[14] for Hungary also establishes a significant socio-economic correlation on account of Clinical research in the country.

- Clinical trials increased the revenue of Hungarian health care providers by US $165.6 million.
- The value of IMPs (Investigational products) was US $67.0 million - meaning that the patients benefited to the tune of this amount, by participating in clinical trials.
- Clinical trial operation and management activities generated 900 jobs and US $166.9 million in revenue among CROs and pharmaceutical companies.
- The contribution of clinical trials to the Hungarian GDP in 2010 amounted to 0.2%.

Ireland:

The socio-economic benefits of clinical trials observed in Ireland - are largely on the same line as observed globally i.e. (a) Early access to newer medicine for patients (b) reduction in cost for patients and for payers (c) expedited entry of newer medicines in the geography that also has a downstream positive impact on societies and government in terms of better healthcare scenario (d) Economic benefits, direct, indirect and induced.

A sample of these benefits are outlined in a study[15] that was commissioned by Cancer trials Ireland and was reported by DKM Economic consultants in 2016. Highlight of the socio-economic impact outlined in the reports are as below:

- Cancer Trials Ireland projected an income of Euro 7.5 million in 2016 - nearly half (Euro 3.06 million) was contributed by the Exchequer.
- For some hospitals, 1 Euro received in grant funding, resulted in attracting an income of Euro 3 from Industry for trials. This made the hospital institutions financially stronger and hence more able to support patient care.
- Drugs savings directly to HSE (Ireland's Health services) from alone cancer clinical trials was to the tune of Euro 6.5 millions. Additional saving was in form of cost of experimental drugs, cost of avoided treatments, improved health and longer lives for patients and downstream benefits in terms of less health burden for future.
- Clinical trial activities added a total of Euro 16.5 million to Ireland's GDP and a revenue to the exchequer of Euro 5.8 million per annum.

Italy:

While there have not been an extensive research conducted in Italy to study the Socio-economic impacts/ benefits of clinical research, Ipolitti et al[16] conducted a retrospective cost analysis on all patients with MPM (Malignant Pleural Mesothelioma) who were admitted between 2014 and 2015.

Result suggested a significant decrease in cost of treating first line patients, where cost of chemotherapy is relevant. Results suggested that the expected reimbursed fee to care for a patient with MPM was approximately Euro 18,214.99. This amount was reduced to Euro 320.18 only.

It might be very interesting to review the impact on a much wider scale in Italy, considering the size of the country and pharmaceutical market.

Thailand:

Clinical research industry represents a significant source of innovation and economic prosperity for Thailand. A study[17] commissioned by Pharmaceutical Research and Manufacturer Association (PReMA) and conducted by Deloitte brought out some interesting details on how the Clinical research is having an impact on Thailand.

- In 2015, a total of USD 320 million were spent on Clinical trials in Thailand. Of these, about USD 120 million were spent by Industry.
- About 111,000 Thai people participated in clinical trials in 2015.
- Every dollar spent on Clinical research in 2015, yielded a return of dollars 2.9.
- About 8,905 people were employed in conducting clinical research. This resulted in an economy of USD 150 million towards remuneration of these staff.
- Additionally, another 6,604 employees indirectly contributed to the clinical research activities.
- Trials contributed to Thailand's GDP to the tune of USD 270 million (representing about 0.05% of GDP).
- For trials conducted in 2015, expected net economic return from medicines produced was estimated at USD 13.4 million.

The above study also came out with clear recommendation on the policies to be adopted to get more clinical research in Thailand.

United Kingdom:

UK represents the developed market for Pharmaceutical Research, Manufacturing and Consumption. Though there are no studies conducted to represent entire UK market, KPMG published a report[18] in 2016 that selectively estimated the economic impact created by Clinical Research Network of National Institute of Health Research (CRN-NIHR).

It is important to note that the impact shown below is only for clinical research activities conducted by NIHR-CRN. The overall impact would be larger - if we consider all other research activities being conducted in the UK.

- The report estimates that in 2014/15, CRN supported clinical research activities generated GBP 2.4 billion of GVA -Gross value added and about 39,500 jobs in the UK.
- Additionally, CRN got an additional revenue & cost saving of GBP 192 million.

While the above study was initiated with an objective of taking a stock of the current situation, it also has resulted into an action plan that intends to proactively measure all aspect of economic activities at CRN level, NHS level, Hospital and site level, to better estimate the same.

With a large market size and mature eco-system for conducting Research, impact of these size are bound to have downstream impact on society and government and entire healthcare chain.

USA:

Being the largest market for Pharmaceuticals and at the forefront of cutting edge research, the stakes involved are quite high for USA. This naturally will have a direct impact on the healthcare providers, payers & patients. PhRMA commissioned a study with Battelle Technology Partnership practice in March 2015 - to understand and estimate the economic impact of clinical trials on state economies. The report "Biopharmaceutica Industry-sponsored Clinical trials: Impact on state economies"[19] highlights following points:

- In 2013 the biopharmaceutical industry sponsored 6,199 clinical trials of medicines in the U.S., involving a total of 1.1 million participants.
- The biopharmaceutical industry spent nearly $10 billion directly in the conduct of clinical trials at the site level across the U.S. in 2013. These amounts are in addition to the significant resources invested in clinical trial-related activities occurring outside the individual trial sites, either within biopharmaceutical company facilities or by their contractors and vendors.
- When considering the overall impact of site-specific clinical trial activity across states, i.e., the ripple effect of expenditures by clinical trial vendors and contractors and spending by industry and vendor employees, biopharmaceutical industry sponsored clinical trials generated a total of $25 billion in economic activity in communities throughout the U.S.
- The five states with the highest number of active clinical trial sites were California (3,111), Texas (2,799), Florida (2,571), New York (2,476), and Pennsylvania (1,972).

The report provided granular details of how respective state economies were positively benefitted from the clinical research.

India:

India's journey on Clinical research has been turbulent - to best describe the phenomenon. It started in late 1990s when a few multinational Pharma companies and CROs came forward to start conducting clinical trials as per ICH-GCP standards. Ironically, most of the medicines which were not available in India - got their Marketing approvals in India without any trials in Indian population (based on global data).

As the field of Clinical trials kept growing in India, the regulatory framework kept maturing better and better (which may also be considered a very positive impact of clinical trial on Indian population). This applied not only to the regulations pertaining the clinical trials, but on all aspects of Drugs control in India.

Between late 1990s and early 2000s, Clinical trial activities grew very well because of a positive regulatory mechanism for approval. However, the monitoring of clinical trials did not reciprocate the ease of getting approval and hence the activities came under a lot of criticism.

Late 2000s and early 2010s - the sector struggled a lot with credibility issues both with the industry fraternity that was conducting clinical trials, but also the regulatory agencies which were monitoring and approving the same. A significantly negative perception was built up against clinical trial activities, as a result of unfounded activism and catchy media highlights.

Between the conducive decade of 1990s and 2000s - the industry grew a reasonable capacity for conducting clinical trials. Below are some approximations (personal view of the author):

- About 20,000 employees directly associated with the conduct of clinical trials
- Another 15,000 employees working to support activities related with Clinical trials.

When the industry came under a lot of challenges and regulatory restrictions, business for many companies suffered and some of them ended up winding/scaling down their business. This resulted into downstream negative impact on the economy, by way of reduced spending by employees. Unfortunately, it is very difficult (if not impossible) to quantify this impact as it was not having any proactive support from the Government, neither it itself had developed meaningful industry bodies that accurately measured & tracked the economic activities.

Last 5 years for the industry are seeing a measured, steady turn-around for the industry and little growth is seen. However, the momentum built in the previous decade (and the expertise matured over time) was lost.

When the Industry faced local regulatory challenges (incidentally, this came immediately after the global recession of 2008), many companies started re-aligning their operations and cross-utilizing India based resources for global off-shored activities. This helped the industry in multiple ways (a) it avoided a huge job loss when the sector was scaling down(b) it avoided a significant economic loss that would have ensued while the trained professionals had to change their field of specialization and re-align their service offering and (c) it started the phase of KPO activities in Pharma R&D, clinical trial trained resources started doing activities like global DM, Medical writing, Pharmacovigilance, back-office ops for Clinical etc.

What next:

The benefits outlined for most of the countries fall into two classical area (a) Benefits to the healthcare sector, early access to medicine, faster clinical development, better care and upgradation of healthcare service standards, many more...(b) Economic benefits - direct, indirect, induced.

In whatever way that we try to comprehend the socio-economic impacts of clinical research, it does point to the need to facilitate these activities and strengthening the sectors. Many countries worldwide are already doing this and others are following suit.

For India, we are clearly sitting at a junction, where any further delay (to attract more clinical research) will push the country behind, in terms of attaining the benefits on clinical research already done so far. While not immediately visible, any delay in strengthening the sector will delay the launch of newer medicines in long term and this will have serious social and economic impact on future generations.

Author has been associated with international Clinical development for more than 20 years and has been founder of an R&D Management Organization (RMO).

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REVIEW ARTICLE

ETHICAL PRINCIPLES AND PRACTICES IN CLINICAL RESEARCH: A BRIEF HISTORY AND GUIDELINES OF CURRENT RELEVANCE

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Abstract

Ethics is the science of moral values and standards. Scientific experiments, especially those in human subjects, need to be guided by specific ethical values in several aspects. Human experiments of newly discovered medicines or medicines under development, the clinical trials, have a fairly healthy ethical history in ancient and medieval times. However, between World War I and 1970s, the history of clinical trials is very disappointing and has often violated the ethical principles. As a result, several guidelines on ethical conduct of clinical trials have been formulated by various stakeholders covering more or less the same principles of ethics. The Nuremberg code, Declaration of Helsinki, the Belmont report, the ICH GCP guidelines, and the ICMR Guidelines on Ethics are some of the examples. All these documents have covered mainly three aspects of ethics of conducting research in human patients or subjects: a) the patient related aspects (protection, justice and beneficence); b) scientific standard and quality related aspects and c) documentation, publication and promulgation related aspects.

Keywords: Ethics, human subjects, clinical trials, guidelines

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Introduction

The word "ethics" finds its root in an ancient Greek word – *êthos* (ἦθος), which means character, moral nature, habit or custom. It has to also do with aesthetics or beauty – again relating to some purely human values. Ethics thus synthesizes, recommends, defends and sometimes re-defines the right moral conduct from the wrong one.

In last hundred years or so, medical, biomedical and pharmaceutical sciences have seen unprecedented advancement. And obviously this advancement was possible because of intense research and development activities. We would limit our focus in this paper to the ethics of clinical research in the aforesaid domains of knowledge.

It is kind of strange that the history of research involving human patients in ancient and mediaeval times of medicine and pharmaceuticals is relatively straightforward and mostly ethical. It is the stuff that has been done in the name of clinical research between World War I and late 1970s that is very bleak and shameful.

Earliest Mentions of Ethics in Ayurvedic Literature

Ayurveda (Charaka and Sushruta), among the ancient treatise, mentions the ethical principles of a medical or surgical practitioner which can be extended to the research in vogue at that time.[1,2]

While recommending ethics in practice, which was an integrated part of {long-term) research then, the Ayurveda says, "*'Having finished his studies… he should go about… with undeluded mind and with his eyes looking straight before him. He must be genial and take the initiative in a conversation. He must never resort to the patient's house uninvited.'*

'He should not administer the medicine in the wrong order nor should he delegate the responsibility to another… He must be versed in the knowledge of characteristics of constitution, drugs, disease and age.'[1]

Regarding the compensations to a qualified physician or researcher, the Ayurveda says, *'This science of life is permanent and yielding meritPractice of medicine is never fruitless, it sometimes gives money, sometimes religious merit, sometimes renown or sometimes the opportunity for study… .'*

In dealing with women, it says, *'His attitude to women should be particularly aloof and detached. When he enters a patient's house he should keep his head bent and not be curious about things and persons about him. If he has to enter to treat a woman, he should never go unaccompanied and he should never laugh nor smile nor exchange irrelevant words with her…. He should accept nothing from the woman without the knowledge of her husband. He should never enter without informing beforehand. He should neither talk nor sit with a woman in privacy. He*

should never look at her when she is uncovered nor laugh at her… .'

Finally, the Ayurveda insist that medicine is a lifelong study. It says that there is no limit at all to the Science of Life. So, further it says to the physician-investigator, '*So thou shouldst apply thyself to it with diligence. This is how thou shouldst act. Again thou shouldst learn the skill of practice from another without carping. The entire world is the teacher to the intelligent and foe to the unintelligent. Knowing this well, thou should listen and act according to the words of instruction of even an unfriendly person, when they are worthy… .* 'The ideal physician is the one who is well-born, of wide learning, of wide practical experience, skilful, pure, practised of hand, self-controlled, fully equipped with all the appurtenances (of healing), in full possession of his faculties, conversant with the normal course of nature, able to take prompt and appropriate decisions.[1,2]

Medical Ethics in Ancient and Medieval Europe

It appears that the medical licensure, guilds, universities, and a reciprocity of obligations and regulations thereof began in Europe in as early as 11th century CE. Far before that epoch, there were many references to medical ethics in ancient Greek and Roman texts. The Hippocratic Oath, for example, is well known in ancient Greek medical texts in its original form between fifth and third centuries BC. Greek and Roman medical literature also allude to the choice of treatment, patient cooperation, confidentiality and information given to the patient.[3]

In particular regards to the relationship with the patients, Desiderius Erasmus' views on medical ethics over 500 years ago is amazingly fitting even to the present day standards. He advocates for reciprocal attention to the patient's duties as well as those of the physician's. By treating this reciprocal relationship as a friendship between extreme unequals, Erasmus was able to maintain the nobility of the medical artandat the same time deal with the culturally sensitive issue of physicians'compensation. It is felt that as Erasmus' treatment of physician-patient reciprocity arose from a classical conception of friendship, there may be grounds for reconsidering the role of friendship in other discourses on medical ethics.[4]

Atrocious Violations of Medical Research Ethics during World War II and Following Few Decades

So called "medical experiments" were performed on thousands of concentration camp prisoners during World War II. These experiments included deadly studies and tortures such as injecting people with gasoline and live viruses, immersing people in ice water, and forcing people to ingest poisons.[5]

After the war was over, in 1947, a War Crimes Tribunal was set up at Nuremberg, Germany. The indictments included

conspiracy to commit crimes against peace; planning, initiating and waging wars of aggression; war-crimes and crimes against humanity. Out of 23 physicians and administrators who were accused, 16 were found guilty and imprisoned and 7 were sentenced to death. In the Tribunal's August 1947 verdict, a section called "Permissible Medical Experiments" became known as *the Nuremberg Code*. A summary of the ethical principles of the Nuremberg Code is provided in Table 1.[6]

The Tuskegee Syphilis Study from 1932-1972; the case of Harold Blauer (1953) at the New York State Psychiatric Institute; the New York City's Jewish Chronic Disease Hospital study of 1963; the Willowbrook Study of 1963-66 are some of the other examples of blatant violation of ethics and patients' rights.[5]

Table 1. Summary of the ethical principles of the Nuremberg Code

	Patient-Centric
1	Voluntary Consent is essential
2	Should be conducted by avoiding physical/mental suffering and injury
3	Adequate facilities should be used to protect subjects
4	Subject should always be at liberty to stop at any time
	High Quality & Scientific Ethics Centric
5	Should be based on previous animal experimentation
6	Risks should never exceed the benefits
7	Conducted only by qualified scientists
8	Scientist in charge must be prepared to terminate the experiment when injury, disability, or death is likely to occur
	Publication or Promulgation Centric
9	The results must be for the greater good of society
10	No experiments should be conducted if it is believed to cause death/disability

Declaration of Helsinki

After the declaration of the Nuremberg Code in 1947, the World Medical Association (WMA) issued a seminal document on the human research ethics, in 1964, in Helsinki Finland, called the Declaration of Helsinki. It has undergone as many as seven revisions, and the last one was issued in 2013 at the 64th WMA General assembly in Brazil.[7]

In addition to its General Principles the Declaration has clear-cut guidance on Risks, Burdens and Benefits; Vulnerable Groups and Individuals; Scientific Requirements and Research Protocols; Research Ethics Committees; Privacy and Confidentiality; Informed Consent; Use of Placebo; Post-Trial Provisions; Research Registration and Publication and Dissemination of Results; and on Unproven Interventions in Clinical Practice.[7]

Although the Declaration is primarily intended for the Physicians, the WMA encourages others who are involved in medical research involving human subjects to adopt these aforesaid principles.[7]

The Belmont Report

The Belmont Report (1978) summarizes ethical principles and guidelines for research involving human subjects. Three core principles are identified: *respect for persons, beneficence, and justice.* This report was issued by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research of the United States.[8]

ICMR 2017 and 2006: Ethical Guidelines for Biomedical Research on Human Subjects

These guidelines[9] have elaborated 12 general principles of Clinical and Biomedical research:

1. **Principle of essentiality:** research being carried out should be essential for the advancement of knowledge that benefits patients, doctors and all others in aspects of health care.

2. **Principles of voluntariness, informed consent and community agreement:** research participant should be aware of the nature of research and the probable consequences of the experiments. Participants then should make an independent choice without the influence of the treating doctor, whether to take part in the research or not.

3. **Principle of non-exploitation:** research participants should be remunerated for their involvement in the research or experiment. The participants should be made aware of all the risks involved irrespective of their social and economic condition or educational levels attained…either through insurance cover or any other appropriate means to cover all foreseeable and hidden risks.

4. **Principle of privacy and confidentiality:** all the data acquired for research purpose should be kept confidential to prevent disclosure of identity, not be disclosed without valid legal and/or scientific reasons.

5. **Principle of precaution and risk minimisation:** due care and caution

should be taken at all stages of the research and experiment (from its beginning as a research idea, formulation of research design/protocol, conduct of the research or experiment).

6. **Principle of professional competence:** clinical research should be carried out only by competent and qualified persons in their respective fields.

7. **Principle of accountability and transparency:** researcher should conduct experiments in fair, honest, impartial and transparent manner after full disclosure of his/her interests in research. They should also retain the research data for a period required by the regulatory authorities.

8. **Principle of the maximisation of the public interest and of distributive justice:** results of the research should be used for benefit of all humans, especially the research participants themselves and/or the community.

9. **Principle of institutional arrangements:** all institutional arrangements required to be made in respect of the research and its subsequent use. All applications should be made in transparent manner.

10. **Principle of public domain:** results of any research work done should be made public through publications or other means. Even before publication, the detailed information of clinical trials should be made public before start of recruitment via clinical trial registry systems,

11. **Principle of totality of responsibility:** all those directly or indirectly connected with the research should take the professional and moral responsibility, for the due observance of all the principles, guidelines or prescriptions laid down in respect of the research.

12. **Principle of compliance:** All those associated with the research work should comply by the guidelines pertaining to the specific area of the research.[9]

Ethics of Randomization in CTs

Randomized clinical trials pose a number of fundamental ethical questions: a) should they be placebo controlled? b) will control arms get standard treatment at all? can randomized trials of all kinds be cross-over?

Morally sensitive investigators must give careful consideration to these questions. In general, the randomized double-blind clinical trial (either with an active control or, in a few cases, with a placebo control) is ethically justified and the preferred method of demonstrating therapeutic effectiveness and safety. Use of randomized double-blind clinical trials

must assure adequate explanation of the research plan to the patient, the documentation of informed consent, adequate consideration of safety, and an acceptably low risk/benefit ratio.

Conclusions

Our medicines need to be evidence-based. And this evidence of efficacy, safety and quality has to be ethical in addition to being scientifically accurate and valid. History has witnessed that pharmaceutical and medicinal experiments on human subjects had had several departures from ethical standards. Since the publication of the Nuremberg Code, Declaration of Helsinki and similar ethical guidelines, we are trying to give our medicines ethical, scientific and universal legitimacy as a society

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RESEARCH ARTICLE

CONCURRENT ESTIMATION OF LOTEPREDNOL ETABONATE AND LEVOFLOXACIN BY UV SPECTROPHOTOMETRIC ABSORBANCE RATIO METHOD FROM THEIR COMBINED EYE DROPS DOSAGE FORM

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Abstract

A simple, sensitive, rapid, accurate and precise absorption ratio method (Q value analysis method) has been developed for simultaneous estimation of loteprednol etabonate and levofloxacin in combined dosage form. Ratio of absorbance at two selected wavelengths was calculated. First wavelength is absorption maxima of respective drug and second wavelength is iso-absorptive point at which both drugs give same absorbance. Loteprednol etabonate showed absorbance maxima at 269.29 and levofloxacin showed absorbance maxima at 298.5 nm in methanol. The iso-absorptive point of loteprednol etabonate and levofloxacin was found to be at 269.29 nm. Linearity was constructed in the concentration range of 5-25 µg/mL. Promising values of correlation coefficient for LE (R^2=0.998) and levofloxacin (R^2=0.999) proves that method is linear. Furthermore, the method was successfully validated in terms of various validation parameters as per ICH Q2 (R1) guidelines. The developed method was successfully applied for estimation of both loteprednol etabonate and levofloxacin from its eye drops. Mean % recovery indicated that no interference was observed from excipients present in the formulation. Therefore, developed method can be routinely applied for simultaneous estimation of loteprednol etabonate and levofloxacin from its pharmaceutical dosage form.

Keywords: Loteprednol etabonate, levofloxacin, absorbance ratio method

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1. INTRODUCTION

Loteprednol etabonate (LE), chloromethyl (11β, 17α)-17-[(ethoxycarbonyl) oxy]-11-hydroxy-3-oxoandrosta-1, 4-diene-17-carboxylate [1] (Figure 1) is a topical corticoid anti-inflammatory which is used in ophthalmic solution for the treatment of allergic conjunctivitis, uveitis, acne rosacea, keratitis, iritis, cyclitis, and selected infective conjunctivitis [2–5]. Levofloxacin (LV) is (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid[6] (Figure 2). LV, one of the commonly used fluoroquinolone antimicrobials, is the active S-isomer isolated from the racemic ofloxacin. Its antibacterial action is twice as active as the racemate ofloxacin *in vitro*. Because of its excellent antibacterial activity and low frequency of adverse effects on oral administration, LV has been widely used for the treatment of infectious eye diseases [7,8]. The combination comprising LV and LE is available as ophthalmic, otic or nasal pharmaceutical preparations, which is commonly used for treatment of conjunctivitis, keratitis, blepharitis, dacrycystitis, hordeolum, corneal ulcer and ocular infections.

Literature survey reveals that various analytical methods have been reported for the estimation of LE & LV individually. UV Spectrophotometric [9–11] Spectrofluorimetric [12,13], HPTLC [14,15],HPLC [16–20] and UPLC [21] methods have been reported for the estimation of LV, whereas HPLC method [22,23] has been reported for estimation of LE. No analytical method has been reported for estimation of LE and LV simultaneously in pharmaceutical dosage form. Therefore, it was endeavored to develop rapid and simple absorbance ratio methodfor simultaneous estimation of LE and LV from eye drop formulation and to validate the method as per ICH Q2 (R1) guidelines [24] .

2. EXPERIMENTAL WORK

2.1 Chemicals and Reagents

LE (99.9 %) reference standard (RS) and LV (99.9 %) (RS) was provided as a gift sample by Sun Pharmaceutical Industries Ltd. and Ajanta Pharma Limited respectively. Methanol (AR Grade) was purchased from S.D. Fine Chemicals Ltd., Bombay, India. LE& LV Eye Drops were prepared in the laboratory having concentration of 0.5% w/v of LE and 1.5% w/v of LV.

2.2 Instrumentation

A UV-2400, Version 2.21 double beam spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm, and a pair of 10 mm matched quartz cells (Shimadzu, Columbia, MD) was used. An analytical Balance, Citizen CX 220 (Citizen Pvt. Ltd, Germany) of capacity10 to 220 mg was used for weighing. Sonicator (Trans-O-Sonic, Erection & Instrumentation Engineers, Ahmedabad, India) was used for solubilization of drug.

2.3 UV Spectrophotometric conditions

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drugs and iso-absorptive point, solutions of both drugs having concentration 10µg/mL in methanol were scanned using UV spectrophotometer within the wavelength range of 200 – 400 nm against methanol as blank. Absorption curve showed characteristic absorption maxima at 230.7 nm for LE and 298.5 nm for LV. Both drugs showed same absorbance at 269.29 nm, so it was considered as an iso-absorptive point of both drugs. **(Figure 3)**

2.4 Preparation of standard stock solution

25 mg of LE (RS) and LV(RS) were accurately weighed and transferred to separate 25 mL amber coloured volumetric flasks and diluted up to the mark with methanol to produce a stock solution of 1000 µg/mL concentration of both drugs. 2.5 mL of aliquot of both stock solutions was separately diluted to 25 mL with methanol to give each solution having 100 µg/mL concentration.

2.5 Preparation of sample solution from eye drops

Sample solution was prepared by diluting 0.1 mL of eye drops to 10 mL with methanol in amber coloured volumetric flask. From that, 1 mL of aliquot was diluted to 10 mL with methanol, which correspondingly gives 5 µg/mL and 15 µg/mL concentrations of LE and LV, respectively.

2.6 Method Validation

2.6.1 Linearity: Preparation of calibration curve

For construction of calibration curve, 0.5, 1.0, 1.5, 2.0 and 2.5 mL solutions of both the drugs were taken from standard stock solution in 10 mL separate volumetric flasks to get the concentration of 5, 10, 15, 20 and 25 µg/mL for LE and LV, respectively. Calibration curve was constructed by plotting absorbance versus concentration.

2.6.2 Precision

Intraday and Interday precision was determined by measuring the absorbance of both the drugs three times within a day and on three different days, respectively. For this, absorbance of solution of both drugs having concentration 5, 15 and 25 µg/mL was measured and solutions were analyzed as per UV Spectrophotometric conditions.

2.6.3 Accuracy (% Recovery)

The accuracy of the method was determined by calculating % recoveries for LE and LV by standard addition method at three different levels (80, 100 and 120 %). Known amount of standard solution of LE (0.2, 0.25 and 0.3 mL) and LV (0.6, 0.75 and 0.9 mL) were added to pre-analysed sample solution containing 0.25 mL of LE and 0.75 mL of LV in 10 ml volumetric flask. Solutions were analyzed as per UV Spectrophotometric conditions.

2.6.4 Robustness

Robustness was performed on concentration (15 µg/mL) of LE and LV. Robustness of the method was determined by making changes in λ_{max} of both the drugs by ± 4 nm. The % Assay values were calculated and compared with that of the standard.

2.6.5 Limit of Detection and Limit of Quantification

For this determination, Calibration curve for both the drugs was repeated six times. Further from which, LOD & LOQ were calculated using mathematical equations given below:

LOD = 3.3 x σ/S

LOQ = 10 x σ/S

Where, σ = Standard deviation of the Intercept

S = slope of calibration curve

2.7 Analysis of LE and LV in prepared combinedeye drops dosage form

Assay was determined for 5 µg/mL and 15 µg/mL concentrations of LE and LV, respectively. Solution was analyzed as per UV Spectrophotometric conditions.

3. RESULT AND DISCUSSION

3.1 Optimization of experimental conditions for absorbance ratio method (Q value analysis method)

From the overlain spectrum of LE and LV which is shown in Figure 3, the wavelengths selected for analysis were 269.29 nm (isoabsorptive point) and 298.5 nm (λ_{max} of LV). LE was quantified at 269.29 nm and 298.5 nm (equation 1). LV was quantified at 298.5 nm using equation 2 because LE doesn't show any absorbance at 298.5 nm. The absorbance of the standard and sample solutions was measured. The absorptivity values for both standard drugs at the selected wavelengths were employed for determination of Q values. The concentrations of drugs in sample solution were determined by using the following equations.

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a y_1} \quad \ldots\ldots 1$$

$$C_y = \frac{A}{ab} \quad \ldots\ldots 2$$

Where, $Q_m = A_2/A_1$, $Q_x = ax_2/ax_1$ & $Q_y = ay_2/ay_1$. A_1 & A_2 are the absorbance of the mixture at 298.5 nm & 269.29 nm respectively; ax_1 and ay_1 are absorptivity of LE and LV respectively at 298.5 nm; ax_2 and ay_2 are absorptivity of LE and LV respectively at 269.29 nm.

3.2 Validation of the Proposed Method

Linearity: A linear relationship was achieved between absorbance and the

concentration of the both the drugs in the range of 5-25 µg/mL. The correlation coefficients of both the drugs for the developed method were found to be not less than 0.998. The results of linearity are shown in table 1.

Precision, LOD and LOQ: Interday and intraday variation in estimation of LE and LV showed that the RSD was always less than 2% during analysis by developed method. These low RSD values show good precision of the method. The results of precision are shown in table 2 and 3.

Accuracy: Recovery studies were carried out by the standard addition method. The results of recovery studies of both the drugs for the developed method are shown in table 4. The % recovery values were in the range of 98-100%, which shows good accuracy of proposed method.

LOD and LOQ: The LOD and LOQ values for both LE and LV were calculated using the equation. The LOD and LOQ values for both the drugs are reported in table 5.

Robustness: The % RSD in robustness studies was found to be less than 2%. The low RSD value indicated robustness of the method. Results of robustness studies for the developed methods are shown in table 6.

3.3 Analysis of LE and LV in prepared combined eye drops dosage form

The proposed method were successfully applied to determine LE and LV in their combined eye drops dosage form. The results obtained for LE and LV were compared with the corresponding labelled amounts. The assay values obtained are shown in table 7.

The proposed method was developed and validated according to ICH guidelines. The results of validation parameters for LE and LV are summarized in table 8.

4. CONCLUSION

The developed method has linear response in the range of 5-25 µg/mL with correlation coefficient of R^2=0.999 (LE) & R^2 = 0.998 (LV). Furthermore, the method was successfully validated in terms of various parameters as per ICH Q2 (R1) guidelines. The developed method was successfully applied for estimation of both LE and LV from its pharmaceutical dosage form, i.e. eye drops. The results of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the formulation. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of LE and LV. Hence, the method can be successfully used for the routine analysis of LE and LV in combined eye drops dosage form.

5. ACKNOWLEDGEMENT

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Pharma Limited & Sun Pharmaceutical Industries Ltd.for generous gift samples of pharmaceutical grade LE and LV respectively.

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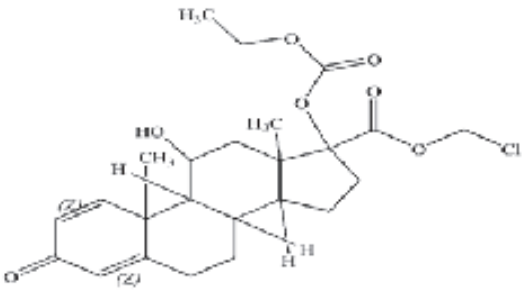

Figure 1: Structure of Loteprednol Etabonate

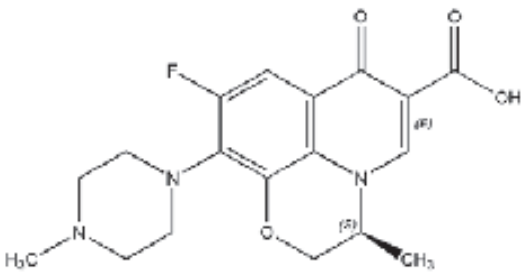

Figure 2: Structure of Levofloxacin

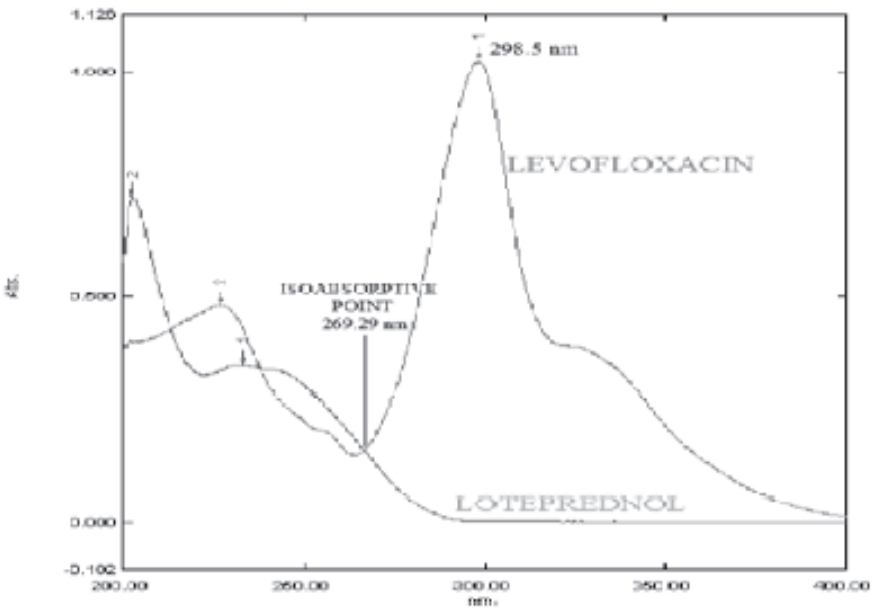

Figure 3: Overlain spectra of LoteprednolEtabonate(10 µg/mL) and Levofloxacin (10 µg/mL) in methanol

Table 1: Linearity of LoteprednolEtabonate and Levofloxacin

Conc. (ppm)	Loteprednol Etabonate		Levofloxacin		Isoabsorptive point	
	269.29 nm		298.5 nm		269.29 nm	
	Mean Absorbance ± S.D. *	% R.S.D.	Mean Absorbance ± S.D. *	% R.S.D.	Mean Absorbance ± S.D. *	% R.S.D.
5	0.1140 ± 0.0019	1.6667	0.531 ± 0.0039	0.8394	0.1136 ± 0.0013	1.1444
10	0.2033 ± 0.0035	1.7216	1.126 ± 0.0116	1.0349	0.2031 ± 0.0034	1.6740
15	0.2935 ± 0.004	1.3628	1.5915 ± 0.0101	0.6392	0.2932 ± 0.0039	1.3301
20	0.3901 ± 0.0051	1.3073	2.193 ± 0.0068	0.3106	0.3896 ± 0.0060	1.5400
25	0.4891 ± 0.0091	1.8605	2.7411 ± 0.0096	0.3517	0.5002 ± 0.0083	1.6593
Linearity Equation	y = 0.018x + 0.017		y = 0.109x - 0.009		y = 0.019x + 0.000	
Correlation Coefficient	0.9989		0.998		0.998	
Slope	0.018		0.109		0.019	
Intercept	0.017		-0.009		0.000	

*** n = 6**

Table 2: Intraday precision of Loteprednol Etabonate and Levofloxacin

Lotepredno lEtabonate

Conc. (ppm)	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	5.15 ± 0.075	1.45
15	14.89 ± 0.205	1.37
25	24.86 ± 0.271	1.09

Levofloxacin

Conc. (ppm)	298.5 nm		269.29 nm	
	Mean conc. (ppm) ± S.D.*	% R.S.D.	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	4.99 ± 0.025	0.50	5.08 ± 0.100	1.96
15	15.00 ± 0.108	0.72	15.18 ± 0.175	1.15
25	25.00 ± 0.055	0.22	25.02 ± 0. 150	0.59

* n=3

Table 3: Interday precision of Loteprednol Etabonate and Levofloxacin

Lotepredno lEtabonate

Conc. (ppm)	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	5.01 ± 0.072	1.43
15	14.99 ± 0.170	1.13
25	24.94 ± 0.346	1.38

Levofloxacin

Conc. (ppm)	298.5 nm		269.29 nm	
	Mean conc. (ppm) ± S.D.*	% R.S.D.	Mean conc. (ppm) ± S.D.*	% R.S.D.
5	4.99 ± 0.028	0.56	5.09 ± 0.076	1.49
15	14.96 ± 0.060	0.40	15.30 ± 0.250	1.63
25	24.99 ± 0.041	0.16	25.02 ± 0.217	0.97

* n=3

Table 4: Recovery (Accuracy study)

Lotepredno lEtabonate

Level of Recovery	Sample Conc. (ppm)	Amount of Std. Added (ppm)	Total amount (ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
80 %	2.5	2.0	4.5	4.47	99.33	100.29
	2.5	2.0	4.5	4.51	100.22	
	2.5	2.0	4.5	4.56	101.33	
100 %	2.5	2.5	5	5.04	100.8	100.03
	2.5	2.5	5	4.96	99.2	
	2.5	2.5	5	5.00	100.1	
120 %	2.5	3.0	5.5	5.52	100.36	100.24
	2.5	3.0	5.5	5.47	99.45	
	2.5	3.0	5.5	5.55	100.91	

Levofloxacin

Level of Recovery	Sample Conc. (ppm)	Amount of Std. Added (ppm)	Total amount (ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
80 %	7.5	6.0	13.5	13.45	99.63	99.98
	7.5	6.0	13.5	13.49	99.93	
	7.5	6.0	13.5	13.55	100.37	
100 %	7.5	7.5	15	15.09	100.6	100.47
	7.5	7.5	15	15.12	100.8	
	7.5	7.5	15	15.00	100.0	
120 %	7.5	9.0	16.5	16.58	100.48	100.32
	7.5	9.0	16.5	16.60	100.61	
	7.5	9.0	16.5	16.48	99.88	

Table 5: Limit of Detection and Limit of Quantification

Drug	LOD (ppm)		LOQ (ppm)	
	298.5 nm	269.29nm	298.5 nm	269.29nm
Levofloxacin	0.06	0.15	0.20	0.50
LoteprednolEtabonate	-	0.05	-	0.19

Table 6: Robustness

Conc.(µg/mL)	LoteprednolEtabonate		Levofloxacin			
	Conc. (µg/mL)		Conc. (µg/mL)		Conc. (µg/mL)	
	267.29 nm	271.29 nm	296.5 nm	300.5 nm	267.29 nm	271.29 nm
15	15.55	11.67	15.09	15.08	13.53	17.47
	15.43	11.53	15.11	15.11	13.59	17.39
	15.50	11.49	14.99	14.98	13.66	17.53
Average	15.49	11.56	15.06	15.05	13.59	17.46
S.D.	0.06	0.09	0.06	0.06	0.06	0.07
R.S.D.	0.38	0.81	0.42	0.45	0.47	0.40
%Assay ± S.D.	103.28 ± 0.388	77.08 ± 0.817	100.42 ± 0.427	100.36 ± 0.450	90.62 ± 0.435	116.42 ± 0.402

Table 7: Analysis of prepared formulation

Drug	Label Claim	%Assay ± S.D. *	% R.S.D.
LoteprednolEtabonate	0.5 % w/v	99.86 ± 0.463	0.46
Levofloxacin	1.5 % w/v	99.63 ± 0.234	0.23

* n=5

Table 8: Summary of Validation Parameters

Parameter	LoteprednolEtabonate	Levofloxacin	
Wavelength	269.29 nm	298.5 nm	269.29 nm
Linearity	5-25 µg/mL	5-25 µg/mL	
Equation	y = 0.018x + 0.018	y = 0.109x - 0.009	y = 0.019x + 0.000
R^2	0.99	0.99	0.99
LOD (µg/mL)	0.16	0.06	0.15
LOQ (µg/mL)	0.49	0.20	0.50
Intraday Precision (%R.S.D., n = 3)	1.30	0.48	1.24
Interday Precision (%R.S.D., n = 3)	1.32	0.37	1.36
% Recovery	100.03-100.29	99.98-100.47	
Robustness (% R.S.D., n=3)	0.60	0.44	
% Assay ± S.D. (n=5)	99.86 ± 0.463	99.63 ± 0.234	

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REVIEW ARTICLE

SIGNALLING PATHWAYS AND MOLECULAR TARGETS FOR SKIN CANCER: INVOLVEMENT OF CDK2 INHIBITION

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Abstract:

Presently, accessible drugs in the market for the management of basal cell carcinoma include Fluorouracil, Imiquimod, Vasodegib and for melanoma, drugs are Dacarbazine, Vemurafenib. Impartial of contemporary training was to improve novel sequences of molecules which would turn on the CDK2 receptors and which might support in the preclusion of the skin malignancy. Malignancy triggering environmental exposures contains substances, such as the elements in tobacco smoke, besides radiation, such as ultraviolet rays from the sun. Skin cancer is one of the malignancies which happens due to the exposure to UV radiation and can prime to very hazardous effects in normal healthy body. Presently, a predictable 9,320 persons will expire due to melanoma in the U.S. in 2018: of persons, 5,990 will be Male and 3,330 will be Female. Only 20 to 30 percent of melanomas remain originate now present moles, although 70 to 80 percent ascend on outwardly regular skin. There are numerous molecular pathways for skin cancer such as, hedgehog pathway, PI3K/Akt pathway, p53 pathway, CDK4/CYCLIN pathway and MAPK pathway, have FDA affirmed medications accessible. Just about 10% of all melanomas are hereditary, and genetic analyses have linked the susceptibility of melanoma to the CDKN2A gene. There are numerous novel targets on origin of melanoma treatment such as Arginine Depletion and Laminins as a novel target. CDKs show significant parts in controller of cell-division then restrained transcript now answer toward numerous intracellular also extracellular signals. There are three kinds of CDK inhibitors such as, ATP competitive inhibitors, ATP non-competitive inhibitor and Allosteric inhibitor.

Keywords: Skin cancer cell division, apoptosis, CDK, ATP

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SKIN CANCER:

Skin tumour is solitary of the predominant among fair or light skinned population. Here are dual kinds of skin malignancy: melanoma and non-melanoma skin malignancy. Among them, melanoma was in charge of the mortality, which can likewise causes unfriendly physical and mental changes in the patients. Every year, nearly 132,000 people were diagnosed for the deadly melanoma cancer. The survival rate is 5-years for the patients with melanoma of about 98% and fifth peak mutual kind of skin tumour among men in United States. Also, the melanoma happens ordinarily in the lower legs, head, neck region of women and for men, it appears on the trunk. It has tendency to happen at a more youthful age, when contrast to the old people with above 57 ages. UV radiation is separated into three radiations that are UV-An, UV-B and UV-C. UV-A are long wavelength and low vitality radiation that causes the tanning and harms the skin cells when uncovered for longer timeframe. UV-B is long wavelength and high vitality Radiation that causes sunburns, photograph maturing and are for the most part in charge of skin tumour. UV-C does not go through the world's climate and consequently are not in charge of tumour advancement. UV radiation causes the direct cell harm by harming the DNA by arrangement of cyclobutane pyrimidine dimers, causes transformation, and expands the oxidative pressure and fiery reactions. Change of p53 quality by UV radiation causes the starting the skin disease. Basal cell carcinoma is a kind of the cell intimate the skin that yields novel skin cell as ancient permit on. Squamous cell carcinomas are thin, plane cells that make up epidermis, or outermost coat of skin. Squamous cell carcinoma is likewise observed to be regular in individuals with more attractive skins, with spots and sun harm. Squamous cell influences the face, the edge of the ears, scalp and lips, especially the lower lip, which is more presented to the sun. Basal cell carcinoma: They frequently apppears as waxy bump, however it can yield extra methods. And arises peak regularly on zones of skin that remain repeatedly unprotected to sun, such as your face plus neck. Squamous cell carcinoma: Epidermis consists of epithelial cells and the cancer of epithelial cell is known as squamous cell carcinoma. It is common on head neck which occurs due to human papilloma virus which is oncogenic. Melanoma: Melanocytes are present in the skin which consists of melanin which is responsible for skin color. Cancer of melanocytes is known as melanoma. Young generation is more affected by this type of cancer.

PATHOPHYSIOLOGY OF SKIN CANCER:

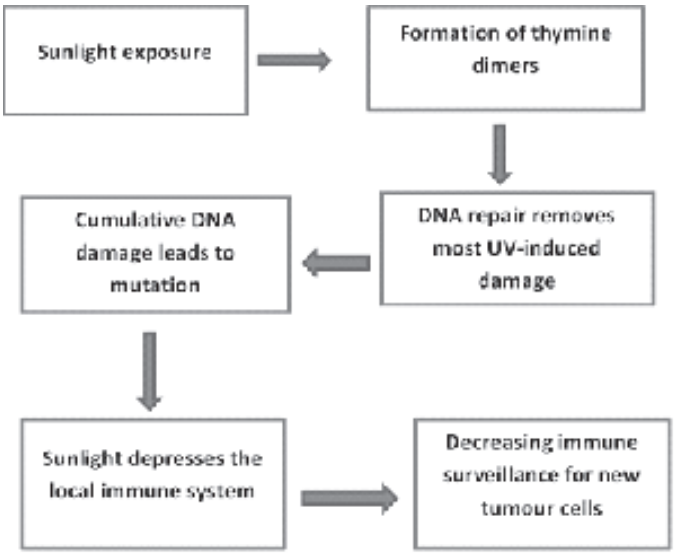


Figure no. 1: Pathophysiology of skin cancer

MECHANISM OF SKIN CANCER:

A portion of UV radiation after the sun or sunbeds can damage the genetic substantial (the DNA) in your skin cells. In the occasion that sufficient DNA damage grows after certain period, it can create cells initiate attractive wild, which can quick skin cancer. UV shafts harm the DNA of skin cells. UVB beams have marginally more vitality than UVA beams. They can harm skin cells' DNA straight forwardly, and are the primary beams that reason sunburns. Coordinate DNA harm can happen when DNA specifically assimilates an UVB photon, or for numerous dissimilar motives. UV sunbeams can like-wise damage the eyes as above 99% of UV radioactivity is expended by forward-facing of eyes. Corneal damage, waterfalls, and then macular degeneration remain on whole believable incessant influences from UV overview then can eventually quick visual absence. Melanoma, category of skin malignancy, can like-wise make inside the eye. Positive (advantageous) impacts of UV. Activates vitamin D – UV after the Sun is required by our figures to create vitamin D. Squamous-cell Basal cell disease matures progressively and can damage the tissue everywhere it yet is perhaps not going to feast too distant off regions or else outcome in expiry. It regularly displays up as an unforced higher zone of skin that potency be gleaming through skin disease will probably spread. It typically introduces as a hard knot with a layered best yet may likewise shape an ulcer. Melanomas are the greatest forceful. Ciphers incorporate a infiltrator that has reformed in estimate, figure, shading, has unpredictable ends, has other than unique shading, is irritated or drains.

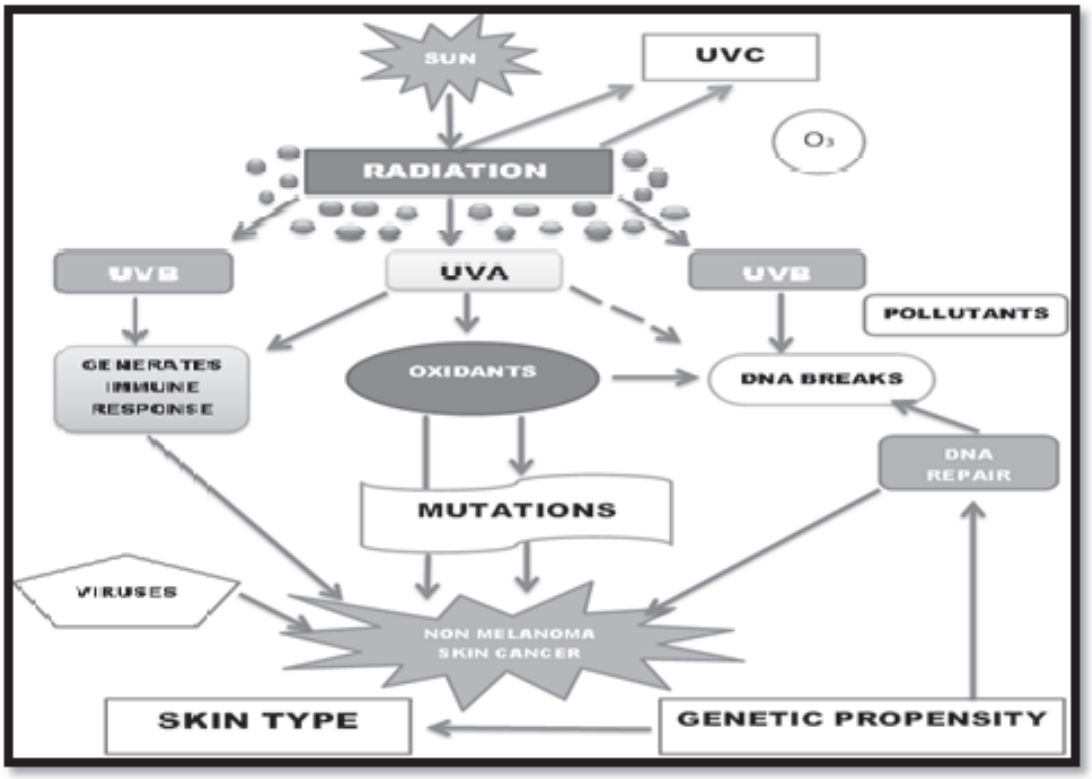


Figure no. 2: Mechanism of Skin Cancer

MOLECULAR PATHWAYS FOR SKIN CANCER:

PI3K/AkT PATHWAY: Like the MAPK pathway, the PI3k/AkT pathway can likewise be initiated by Ras. Once enacted, downstream effectors of Akt square apoptosis and advance cell multiplication and intrusion. despite the fact that PI3K pathway are accepted to be uncommon, downstream parts of the PI3K/AkT pathway relentlessly increment amid melanoma movement, and are changed in half to 60% of melanomas.

P53 PATHWAY: P53 is a cell protein that manages apoptosis, cell expansion and DNA repair. The TP53 tumour silencer quality, which codes for p53, is transformed in roughly half of BCCs. Transformed p53 is additionally the most widely recognized hereditary variation from the norm found in SCC. The nearness of transformed p53 in SCC starts from actinic keratosis (AK), an incessant antecedent to SCC. AK lesions regularly keep changed p53, and those transformations stay as AK advances to SCC.

CDK4/CYCLIN D PATHWAY: Approximately 10%of all melanomas are hereditary, and genetic analyses have linked the susceptibility of melanoma to the CDKN2A gene. Hereditary CDKN2A mutations have remained confirmed in

25% to 50% of relations with genetic melanoma and in at least 10% of patients with numerous crucial melanomas. Somatic CDKN2A mutations have been described in 30% to 70% of irregular melanomas.

MAPK PATHWAY:

Motioning through the MAPK pathway is pivotal for the multiplication of melanocytes, the sound shade cells that offer ascent to melanoma. Nonetheless, hyper-actuation of the MAPK pathway is found in more than 90% of melanomas with roughly half of all patients showing mutation's, in the kinase BRAF, and around 28% of all patients harbouring transformations in the MAPK pathway up-stream controller NRAS.

HEDGEHOG PATHWAY: Specifically, the basic part of hedgehog motioning in the advancement of basal cell carcinoma has remained forcefully exhibited by hereditary transformation examinations mouse prototypes of basal cell carcinoma, and operative clinical hearings of basal cell carcinoma utilizing hedgehog flagging inhibitors. This pathway is one of the significant controllers of cell enlargement and separation amid embryogenesis and early improvement.

TARGETS FOR SKIN CANCER TREATMENT:

The essential mechanisms of the Hippo-signalling pathway are force of kinases that rule phosphorylation of down-stream transcriptional co-activators, specifically, YES associated protein(YAP) besides W-W domain-containing transcription regulator protein-1 (WWTR1, likewise identified as TAZ). The Hippo-signalling pathway remains significant tumour suppressor pathway, besides its deregulation has been well-known in change of human tumours, in which YAP or WWTR1 permit malignant cells overawed contact inhibition, besides to produce then feast irrepressibly. Stimulatingly, though, current educations must said a some-what dissimilar nonetheless possibly extra fascinating YAP or WWTR1 story, as these educations originate that YAP or WWTR1 purpose as dominant centre that participates signals after numerous up-stream signalling, cell-cell interactions besides mechanical forces besides then fix and trigger dissimilar down-stream transcriptional influences direct cell community behaviour then cell–cell interactions. In this evaluation, we extant newest results on character of YAP or WWTR1 in skin physiology, pathology besides tumorigenesis then deliberate positions of a new advanced therapeutic interferences that mark YAP or WWTR1 in human malignancies, in addition to their forecasts for usage as skin malignancy managements. Proliferating keratinocytes in basal cell layer probable involve nuclear YAP besides WWTR1 expression for preservation their proliferation besides inhibition of their terminal differentiation, as damage both of these proteins in basal layer of mouse skin

suppresses cell-proliferation, foremost to hair

damage besides reduced wound remedial. It has been exposed that YAP ablation outcomes

differentiation besides increased apoptosis in cultured mouse keratinocytes. In difference,

nuclear YAP up regulation stimulates cell proliferation besides suppresses mouse basal

epidermal keratinocyte differentiation, important to epidermal hyper thickening besides hair

follicle invagination.

TREATMENT FOR SKIN CANCER:

Excisional surgery: The doctor utilizes a surgical tool to expel the whole development and encompassing fringe of evidently ordinary skin as a wellbeing edge. Electro-surgery: This method is typically held for little sores. The development is scratched off with a curette, and consuming warmth created by an electro-burning needle devastates remaining tumour and control dying. Cryosurgery: solidifies (with fluid nitrogen utilizing cotton tipped implement or splash gadgets) the influenced skin and the dead skin cells tumble off. Radiations: searches out and crushes disease cells with radiation, and called radiotherapy. Radiation utilizes high vitality beams to harm malignancy cells and stop them from developing. Photodynamic therapy: It can be particularly helpful for developments on the look and scalp. A compound operator that responds to light, for example, current 5-aminolevulinate, is connected to Development and taken up by anomalous cells. Laser surgery: This treatment isn't yet FDA affirmed for SCC; however it can be utilized for shallow sores, through repeat tolls like those PDT.

CYCLIN DEPENDENT KINASE PATHWAYS:

Cyclin dependent kinase (CDK) are the protein kinases which are considered through challenging a dispersed subunit cyclin, transports shires indispensable for enzymatic movement. Cdks display remarkable portions in organizer of prison cell departure also rational transcription now answer toward numerous intracellular also extracellular signs. Evolutionary development of Cdk domestic now mammals controlled to objectivity of Cdks addicted to thrice cell cycle connected sub-families (CDk1, Cdk5 also Cdk4) also fifth Transcriptional sub-families (CDK7, Cdk9, CDK8, Cdk20 and CDK11). Complementary proto-typical Cdc-28 kinase of auspicious Yeast, thoroughgoing of this Cdks quandary unique otherwise an inadequate Cyclin, trustworthy through purposeful specialism through development. These assessments recapitulate in what way, though CDKs are customarily disengaged into cell-cycle

otherwise transcriptional CDKs, this activity is repeatedly specific in numerous domestic associates. Not amazingly, deregulation of these domestic of proteins remains a trademark of numerous illnesses, encompassing malignancy, besides drug-targeted inhibition of unambiguous associates consumes manufactured same promising consequences in clinical hearings.

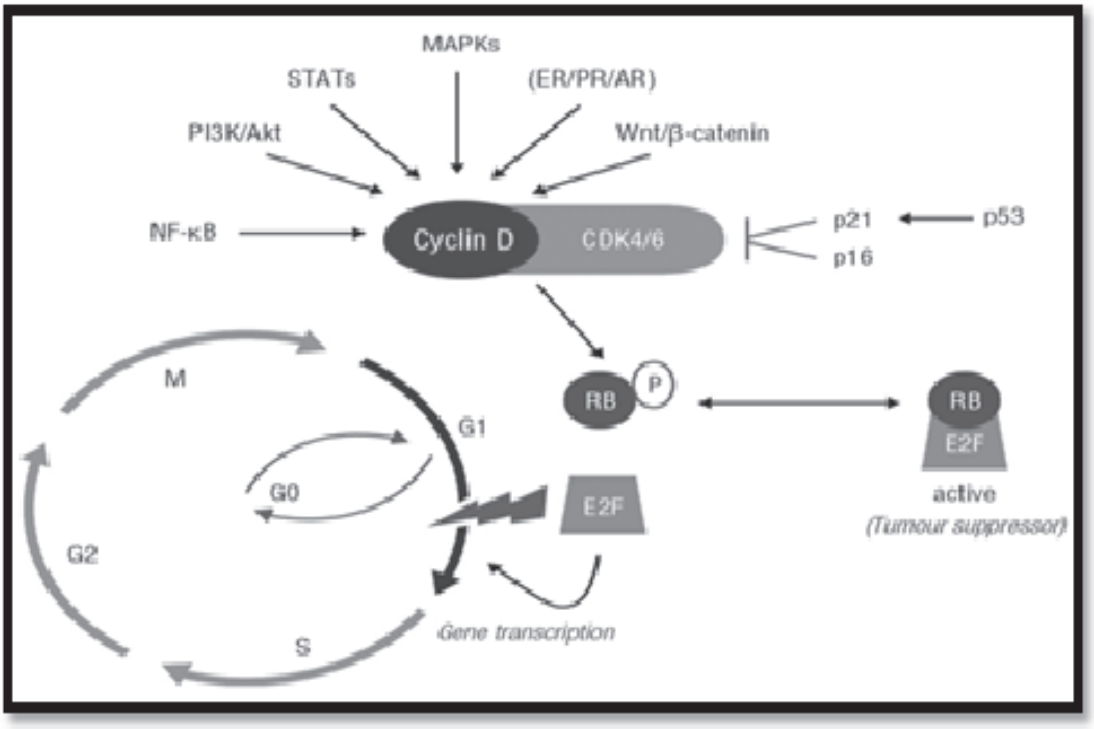


Figure No. 3: CDK Pathway

Table No. 1: CDK Inhibition in Clinical Advance

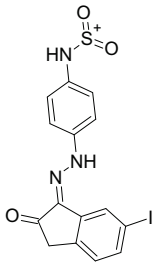
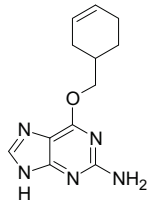
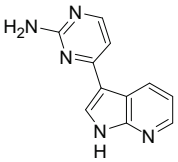
DRUG CANDIDATE	COMPANY	ADMINISTRATION MODE	CDK INHIBITION	CLINICAL TRIAL STAGE
Flavopiridol	Sanofi Aventis	Intravenous	CDK1, CDK2, CDK4, CDK6, CDK7, CDK9	2
Roscovitine	Cyclacel	Oral	CDK1, CDK2, CDK7, CDK9	2
Dinaciclib	Merck	Intravenous	CDK1, CDK2, CDK5, CDK9	3
Palbociclib	Pfizer	-	CDK4(D1), CDK(D3), CDK6(D2)	Approved
Ribociclib	Novartis	Oral	CDK4(D1), CDK6(D2)	3
Abemaciclib	Eli Lilly	Oral	CDK4(D1), CDK6(D1)	3

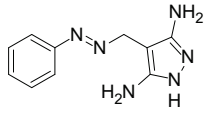
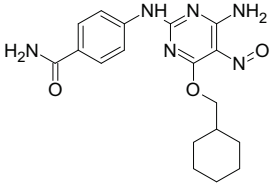
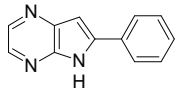
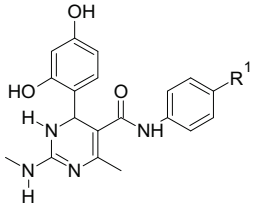
INVOLVEMENT OF CDK2 IN SKIN CANCER:

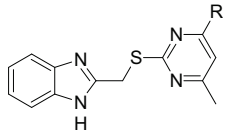
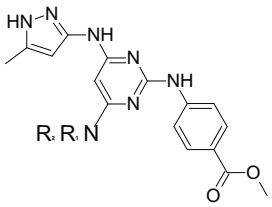
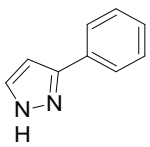
P53 protein is contemporary in multifaceted with the mdm in cytoplasm. As soon as one is unprotected to UV radiation, the protein disconnects and enters into the cytoplasm, combines with DNA and generates to the p-21 protein which is natural inhibitor of CDK2/cyclin A. hence the cell cycle gets intermittent and the growth of abnormal cells stops. But when one is unprotected to unnecessary radiation then DNA gets transformed and the pathway stops which results in the growth in the tumour. Generally DNA involve of purine-pyrimidine base paring, due to unnecessary radiation pyrimidine-pyrimidine dimers are designed. In addition to that UV radiation also persuades other types of DNA injury such as cytosine photo hydrates, purine photo-products. UV radiation causes DNA injury by constructing sensitive oxygen classes such as superoxide anion, singlet oxygen and hydrogen peroxide through endogenous photo sensitizer. Extremely irritable and small lived molecules yield only strand cessation or DNA protein cross linked, different sources in DNA.

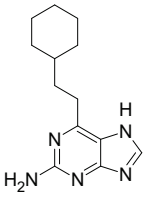
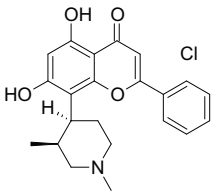
MOLECULAR MOEITY IN CDK INHIBITION:

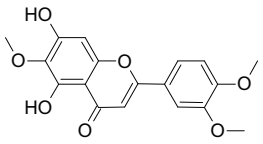
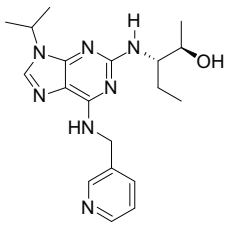
Table No. 2: Molecular Moeity in CDK Inhibition

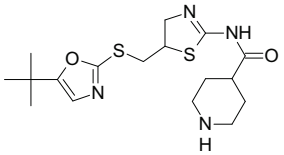
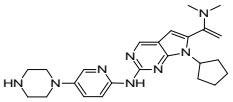
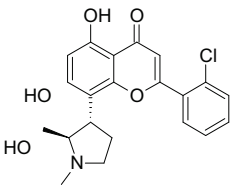
STRUCTURE	AUTHOR	DESCRIPTION
Oxindole Moeity as CDK2 Inhibitor: 	Bramson et al.	Described as oxindole moiety for the inhibition of CDK2. He originate '1H indole -2,3-dione-3-phenyl-hydrazones and 3 -(aniline-methylene)-1,3-dihydro-2H-indol-2-ones' most active Compound in the sequence molecules which are described in the series use to avoid the alopecia which happened due to chemotherapy.
Guanine as CDK2 Inhibitor: 	Morales et al.	Approved out the computational study on the guanine derivative which are previously synthesised in which they carried out docking study by AUTODOCK -3.0 rank the mixtures in reducing value of potency.
Meriolins-(3-(Pyrimidin-4-yl)-7-azaindoles) as CDK2 Inhibitor: 	Echalier et.al	Synthesised : -(3-(Pyrimidin-4-yl)-7-azaindoles) is hybrid assembly of Meridianins then variolins which are removed from the plant Aplidiummeridianum and sponge Kirkpatrickia Variolosacrosspon-dingly. M erolines show effective activity on the CDK especially CDK2 and CDK4.

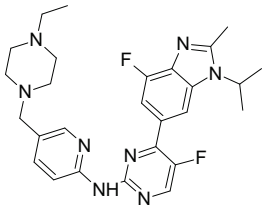
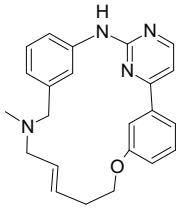
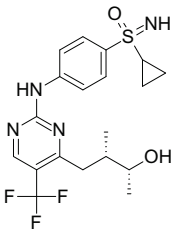
PyrazolMoeity as CDK2 Inhibitor: 	Krystof et.al.	Discover the SAR study on the series of pyrazol and its activity were approved out over the cell line in which he found that 4-((3,5-diamino-1H-pyrazol-4-yl)-diazenyl) phenol moiety more potent proceeding CDK9 as well as on CDK2.
Cyclohexylmethoxypyrimidine as CDK2 Inhibitor: 	A.Ece and F. Sevin	Approved out the QSAR study on the sequence of beyond moiety to find the physicochemical relation -hip amongst the molecules and there pharmacological activity in which they produce the model equation by which we can forecast substitution on the moiety on the structure which can advance the pharmacological activity of the Molecule.
Alosine(6-Phenyl(5H)-pyrrolo-(2,3b) pyrazines) as CDK2 Inhibitor: 	Mettey et.al.	Competitive inhibitor of ATP which interrelates with the amino acid Leu83 hence theatres as noteworthy role in cell cycle capture at G1 then G2 stage. They permitted out the study on the 26 kinases in which they originate that alosin A is supplementary active cdk2/cyclin complex.
Synthesis of Pyrimidine moiety: 	Ibrahim et.al.	Carried out design, synthesis, pharmacological calculation of novel pyrimidine derivative as CDK2 inhibitors in which they approved out the in -vitro cell analysis in human refined cells of malignancy. In the current they also approved out the synthesis of purine, urea and imidazole.

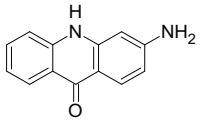
Pyrimidine and benzimidazole Moiety: 	Ju Chen et.al.	Approved out the mixture of the scaffold contain of the grouping of pyrimidine and benzimidazole moiety, pyrimidine moiety having excessive chemical consequence with various pharmacological activity in contradiction of the viral infection, bacterial infection and cytotoxic effect. On the other hand, benzimidazole moiety described for the malignancy management as topoisomerase Inhibitor.
6-substituted pyrimidines: 	Luo et.al.	Described the design, mixture then bio -assessment N-tri-substituted pyramidine dervatives as effective aurora -A-kinase inhibitors. Deliberate compound 6 -substituted pyrimidines on aurora. A Kinase presented excessive activity in contradiction of solid CNE -2 cancer cell and selectively blocked cell cycle Development at the G2/M stage.
3-Aryl Pyrazoles: 	Xianfeng Huang	A new sequence of pyrazole derivative containing hydroxamic acid group were planned and synthesized as multi-target inhibitors targeting CDK2 & HDAC. Pyrazole is a 5-membered ring structure unruffled of 3 carbon atoms and 2 nitrogen atoms in contiguous positions.

8-substituted O6-cyclohexyl methyl guanine as CDK2 inhibitors: 	Benoit Carbain et. al.	Assessment of the properties of purine C-8 subsitution within a sequence of CDK1/2-selective O6 cyclohexylmethylguanine imitative revealed that strength ↓ originally with increasing size of the alkyl substitution.
Imidazole pyrimidine amides as CDK inhibitors:  Imidazole  pyrimidine	Jones CD et. al.	The sequence was originate to have much enhanced CDK2 inhib -ition and powerful in vitro anti-proliferative properties beside malignancy cells. Regulator of overall lipophilicity was important to accomplish good in vitro power along with satisfactory physioche-mical properties and limitations in contradiction of inhibition of both CYP isomers then the HERG potassium ion channel
3-Substitution 4-hydroxycoum-arin as CDK inhibition: for Flavopiridol 	Nehad A. Abdel Latif et.al.	This combined target compounds showed unusually high affinity and selectivity near CDK1B, related to flavopiridol, with Ki values in the short nanomolar series is (ki=0.35-0.88Nm) and flavopiridol is 1 st cohort of pan-CDKs inhibitor with anti-tumour activity credited to down-regulation of CDK -9 tonic ani -apoptotic proteins, exclusively Mcl. Newly, a new generation of CDK has been developed and progressive to phase 3 trials for definite types of malignancy.

3-Substitution 4-hydroxy-coumarin as CDK inhibition: **for Eupatilin** 	Nehad A. Abdel Latif et. al.	Eupatilin, a natural flavone complex removed from Artemisia asiaticaNakai, down -regulation the expression of CD1 in MCF -10-A-ras cells. It produced a reduction in c-Jun appearance and DNA binding action of AP -1 factor Transcription which is an main factor in the cell cycle detention of ras transformed breast epithelial cells.
Activity of CCT068127: **A effective CDK2 and CDK9 Inhibitor:**  (2~{R},3~{S})-3-((9-propan-2-yl-6-(pyridin-3-ylmethylamino)purin-2-yl)amino)pentan-2-ol	Steven R. whitta-ker et. al.	Enhanced from the purine model of seliciclib, CCT068127 shows bigger effectiveness and fuss iness in contradiction of refined CDK2 CDK9 -greater antiproliferative activity alongside human colon malignancy and melanoma cell lines. CCT068127 treatments consequence diminished retino-blastoma, condensed phosphorylation of RNA polymerase 2, and initiat ion of cell cycle seizure and apoptosis. The transcriptional signature of CCT068127 displays greatest resemblance to additional small-molecule CDK and also HDAC inhibitors and CCT068127 produced a affected damage in countenance of DUSP6 phosphate.

SNS032 contain thiazole moiety for CDK2 inhibition: 	Sunesis	SNS032, formerly called BMS -387032. This compound comprise a thiazole unit, selectively hinders CDK2, CDK7 and CDK9. SNS032 is in stage -1 clinical trials for management toward chronic lymphoid leukemia laterally through numerous myeloma, and method of orga nization now intravenous. Pre -clinical studies established that SNS032 remained toward hinder cell cycle movement laterally through transcription.
LEE-011 (Ribociclib) contain activity of Pyrrolo-pyrimidine as CDk Inhibitor: 	Company: Novartis	The main alteration deceits now bicyclic core meanwhile LEE-011 owns Pyrrolo -pyrimidine. Has high selectivity for CDK4 and CDK6 with potential anti -neoplastic activity.
P276-00 (Riviciclib) contain Piperdine and Pyrrolidine moiety: 	Nicholas piramal company	It is analog of flavopiridol . Somewhere piperdine moiety have reformed for a pyrrolidine as well as produced from penetrating structure motion as well co -crystallization exertion. It inhib its efficiency of CDK -9, but also extra Cdks such as CDK1, CDK2, and CDK4. It displays effective anti -proliferative properties against various human cell lines.

LY2835219 (Abemaciclib) contain pyrimidines moiety: 	Eli lilly	It is discerning CDK4 or CDK6 inhibitor, hindering cells at G1 Stage owing to inhibition of phosphorylation of pRb protein. It might manufactured in four step method exhausting Suzuki coupling, shadowed through a 'Buchwald Hartwig' animation th rough last phase existence a reductive amination by the Leuckart reaction.
TG02 (SB1317) contain Pyrimidine based derivative: 	S*BIO	It is pyrimidine built imitative that prevents CDKs composed through JAK2 also FLT3. It persuades G1 cell-cycle seizure as well as apoptosis in comprehensive variety toward tumour cell lines. Primary values toward progenitor cells resultant from acute myeloid leukemia as well as poly-cythemiavera patients are precise complex to TG02.
BAY-1000394 (Roniciclib) contain pyrimidine: 	Bayer	Constrains CDKs elaborate in the cell cycle laterally with the unique concerned in the regulation of transcription with IC50 standards fluctuating from 5 to 25 nM. It studies also exposed that it could be effectual in grouping with cispl-atin. It evidently disables many precincts of further drugs meanwhile shows great solubility in water, uniform by neutral pH, and short Efficacious oral doses. It have Establish anti -tumour mov ement xenograft prototypes unaffected to average medicines such example cisplatin, doxorubicin, and otherwise paclitaxel.

3-aminothioacridinone: 	Kelley's group	It constrains CDK4/cyclinD Multifaceted in communication through site dissimilar from ATP binding site. It also revealed very stimulating in vitro inhibition near CDK4/cyclinD. Unusually, in vitro tests publicized that 3 -aminothioa-cridione had superior inhibitory movement on tumor cells linked to regular cells.

CONCLUSION:

There are numerous novel targets on origin of melanoma treatment such as arginine depletion and laminins as a novel target. CDKs show significant parts in controller of cell division then restrained transcript now answer toward numerous intracellular also extracellular signals. CDK has a critical role in the regulation of cell progression. Higher percentage fraction of CDK-2 positive cells observed in squamous cell carcinoma compared with precancerous lesions may be useful for histopathology diagnostics to this cancer. Cyclin dependent kinases (CDKs) function as central regulators of both the cell cycle and transcription. CDK activation depends on phosphorylation by a CDK-activating kinase (CAK). Since Cdks need to be free of Cdk inhibitor proteins (CKIs) and associated with cyclins in order to be activated, CAK activity is considered to be indirectly regulated by cyclins. Phosphorylation is generally considered a reversible modification used to change enzyme activity in different conditions. We need high quality, long-term randomized clinical trials of the effectiveness of screening on skin cancer.

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REVIEW ARTICLE

ARE GENERIC DRUGS THAT SAFE AND HAVE SAME EFFICACY WITH THAT OF BRAND DRUGS?

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Abstract

A drug is a biochemical substance which has biological effects in the body when ingested or otherwise introduced in the body. Its takes many years to discover a new drug as it is has to undergo several operations, several tests and clinical trials leading to huge cost based on expenditure of the research. The drug is to be marketed and manufactured in pharmaceutical industry only if it has passed the USFDA standards. Once the drug passes the USFDA standard the drug is manufactured, and the discoverer is awarded patent for that same drug for certain period of time. As the drug research is much costly the discoverer sets the prices for the drug which is too high, at least not affordable by common community. For this reason, Generic drugs are introduced in the market. In the recent era generic pharmaceutical are the largest contributor of drugs in the market and are in high demand due to cost effective. As all the generic drugs are approved by USFDA and are allowed to be marketed only when the brand drugs discoverer do not hold the patent for the same drug and the brand drugs is in the market for years and well-established safety profile. Although Generic Medication appear to be same as brand drugs, but variation in manufacturing facilities may lead to unseen adverse events. On other side, generic drugs are tested for bioequivalence properties within a certain range compared to brand discoverer drugs, safety and efficacy testing are not required; therefore, it can be said that generic drugs are not therapeutically equivalent to branded innovator drugs the question of requirements of Pharmacovigilance for generic arises when there is plethora of information available for brand drugs regarding safety and adverse effects. [1] [6]

Keywords: Generic Drugs, USFDA, Pharmacovigilance, Brand Drugs

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INTRODUCTION:

A generic drug is a pharmaceutical drug which is equivalent to a brand-name product in dosage, route of administration, strength, quality, Kinetics, and its intended use. It may also refer to any drug which is marketed under its chemical name without advertising. For getting the approval to market the generic drug an abbreviated new drug application termed as ANDA is to be submitted by the drug companies. The Drug Price Competition and Patent Term Restoration Act of 1984, commonly known as the Hatch-Waxman Act, allowed ANDAs to be possible by making a compromise in the drug industries. Hence, the generic drug gained access to the market for the prescribed drugs and discoverer companies gained restoration of patent of their products. Any new drug is developed under patent protection. This patent protects the investments involved in the development of the drug by allowing the company to have the right to sell the drug while the patent is in the effect. The manufacturer can apply to the FDA for the selling of generic version after the expiration of the patent period. Further, the ANDA process doesn't require the sponsor to repeat test animals, ingredients or dosage forms which are already approved for the safety and its effectiveness. [6] [8]

The use of generic drugs is indicated from many countries in order to reduce medication price. However, some points, such as bioequivalence and the role of excipients, may be clarified regarding clinical efficacy and safety during the switch from brand to generic formulations [6].

A Generic Drug Must Contain following parameters:[4]

- It must contain the exact active ingredient as of the brand innovator drugs.
- It should be replica of dosage form, strength, and route of administration with respect to Brand drugs.
- It should have the same therapeutic uses.
- It should be bioequivalent
- It should meet the same batch requirement for identification, Purity, Strength, Bioavailability.
- It should be manufactured under exact GMP guidelines as of the Innovator drugs.

Although all the components in the generic drugs are same, there might be variation in excipients used in manufacturing or other activity enhancers. Excipients are the substance which are added to the formulations to render the drug in a compatible form for administrations or the stability related factor of drug over shelf life. These excipients are not in active form i.e they do not have any therapeutic activity as such hence they are inactive substance. Another arising factor which

add up to difference in generic from brand drugs is mismatched container system. Recently some parenteral formulations got recall from market due to issues with the final product which was due to mismatched container system. Recently several products were recalled from market by FDA due to problem in the final products.

Some Example of recalls by FDA for safety and efficacy factors are: [2]

Drug
Valsartan/Amlodipine/HCTZ Tablets
Levothyroxine and Liothyronine (Thyroid Tablet USP) 15mg, 30mg, 60mg, 90mg and 120mg
5% Dextrose Injection
Piperacillin and Tazobactum for injection USP 3.375g

Need of Pharmacovigilance for Generic Drugs

Due to high exposure and increasing use of Generic Drugs, its safety and efficacy are very important. At physiological factors, Generic medicines provides the same safety and activity as that of originator drug. The approval of process of Generic drugs do not involve clinical trials as in case of Brand Drugs. So the lack of Clinical Trials makes the need of Pharmacovigilance mandatory for Generic Drugs. Pharmacovigilance is "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem".It has been found that adverse effects reporting for Brand Drugs remained same even after maximum use of generic drugs. In most of cases patients are switched from brand drugs to generic drugs to minimize the medical expense. Due to high reporting of adverse reaction for brand Drugs same applies with their respective Generic Drugs as they are biosimilar drugs with same activity and efficacy. Therefore, better Pharmacovigilance system is required for Generic Drugs. [3]

CASE REPORT – 1 [9]

A 32-year-old man in Jamnagar was visiting his Physician for Acute Bacterial Bronchitis. History described the presence of Blood Hypertension in treatment with ACE Inhibitor (Captopril – 12.5mg daily).On examination blood pressure was found to be 132/89 mm Hg, Body Temperature was around 38 degree Celsius and no other symptoms and adverse effects were observed.Clinical manifestations documented the presence of Short breathing, Cough with Greenish Mucus, and slight sneezing. Therefore, Paracetamol 500mg and Levofloxacin 500mg were prescribed, but after 7 days patient returned to the physician for persistence of Symptoms. A detailed Pharmacological evaluation founded that patient was advised by pharmacist to take

Generic Levofloxacin 500 mg due to lower price available generic Drug.Generic Levofloxacin was changed to Brand Drug which showed complete improvement of symptoms in 4 days and with no major side effects.

CASE REPORT – 2 [10]

A 25-year-old man in industrial area of Rajkot visited his physician for high fever (38°C), with no history of clinical manifestation or other systemic dieses. Some Clinical symptoms showed with little coughing and greenish Mucus and shortness of breath. After diagnosis acute bronchitis was hypothesized and treated with Paracetamol 500mg and Levofloxacin 500 mg was prescribed for 10 days.But unfortunately, patient returned to physician with increased symptoms of Cough and sneezing in addition to Headache and frequent Urination. On detailed diagnosis and evaluation, it was found that patient was using Generic Levofloxacin 500 mg.

Physician advised to use Branded Levofloxacin 500 mg for next days and the symptoms were resolved with no side effects. Physician also reported the Adverse Drug reaction in ADR center.

In the above cases the following things can be noted:

- The use of Generic drugs over Brand drugs or switching to generic Drugs from Brand Drugs was prescribed by pharmacist. The Pharmacist can recommend and sell Generic Drugs against Brand drugs if the doctor does not specifically ask for Brand drugs.

- In both the cases the use of Generic Drugs was limited to 3-4 days which showed no activity or even showed adverse reaction

- In the both the cases use of Generic drugs was only for 3-4 days which didn't show any Therapeutic activity which can be argued that also continuing to next few days the drug might show therapeutic activity.

In these cases a lack of efficacy was observed and during treatment with Generic Drugs can be hypothesized.A number of reasons could be involved in lack of efficacy and development of side-effects such as,

- Difference in excipients:

  Previous studies have suggested that a possible clarification in clinical difference between brand formulation and a generic one, might be characterized by the difference in excipients. The EMA guideline for bioequivalence explains about the presence of excipients that could influence "gastrointestinal transit (e.g., sorbitol, mannitol, etc.), absorption (e.g., surfactants or excipients that may affect transport proteins), *in vivo* solubility (e.g., co-solvents) or *in vivo* stability of the active substance" [11]

- Difference of ±20% of bioequivalence between generic and brand: It is important to highlight that in current law the difference of 20% in bioequivalence is between brand drug and its generic formulation, but it is not possible to outline the bioequivalence during the shift between generic formulations. This difference could play a role in the effectiveness of drugs and it is very relevant during treatment with antibiotic drugs. [12]

- Impurity of pharmaceutical preparation: Quite a lot of studies have shown that generics formulations had a total impurity rate higher to the 3% in comparison to brand formulation. This factor has been previously reported to affect the bioavailability of the drug and therefore, its therapeutic efficacy.[13]

In this manner, the change from brand to generic formulation might not always be considered favorable according to cost-effectiveness.Therefore, the Pharmacovigilance for Generic drugs are to be made mandatory to ensure safety and efficacy of the Generic Drugs.

CONCLUSION:

In country like India, which is still under developing stage and high below poverty ratio, awareness for use of Generic Drugs are to be made. Pharmacovigilance for drugs will continue to provide better safety aspects for both Brand and Generic Drugs. Generic Drugs are to be made mandatory for Pharmacovigilance as they do not involve Clinical trials. The pharmaceutical manufacturer can use low grade Active ingredient or excipient to produce the Generic Drugs leading to lack of efficacy and other side or adverse reactions. Several case reports states that use of Generic Drugs over Brand Drugs leaded to low efficacy and other adverse reaction. Generic Drugs are prepared to minimize the cost of Medicine, but if the Generic Medicine cannot induce or influence the efficacy or therapeutic activity it will eventually increase the cost of medicine. Therefore, the Pharmacovigilance for Generic Drugs are to be made strict and guidelines are to be made for the same.

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REVIEW ARTICLE

BAMBUSHA: REALM OF INDIAN TRADITIONAL MEDICINE

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Abstract

Over the past decades in Indian antiquity, Vanshlochan (Bambusha) is considered as a tonic in treating various ailments. Initial development of such ingredient, predominantly silicon compound, with trace amount of certain elements. A methodological strategy offers to characterize novel candidate bambusha drugs from various bamboo species plants in favor of modern instrumental technique like physico-chemical, X-ray diffraction, fourier transform infra-red spectroscopy, scanning electron microscopy, inductive coupled plasma atomic emission spectroscopy. Bambusha is recommended as a bioavailability enhancer and provide synergistic effect like various well-known medicaments - Sitopaladi, Talisadi, Dadimashtaka, Vilwadichurna and Prabhakar, brambhivati in Ayurveda along with other Unani formulation. The safety and efficacy of this mad stone is not explored scientifically.This review mainly focuses on the traditional, experimental, physico-chemical information of Bamboo manna.

Keywords: Ayurvedic, Bambusha, Silicon, Synergistic, Bioavailabity.

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1. Introduction

Bambusha also known as Bamboo-manna, Tabasheer, Vanshlochan and Eye of the bamboo is the siliceous materials generally found in the culms of the some species of bamboo. It is one of the drug of choice for treating bronchitis, asthma, emmenagogic, febrifugal, demulcent, poisoning cases, paralytic complaints, cardio-tonic, aphrodisiac, small pox and measles, pecotoral properties and haemostatic etc [1,2]. It is also used as one of the valuable ingredient in various Ayurvedic formulation like Sitopaladi, Talisadi, Dadimashtaka, Vilwadichurna, Chawnprash [3]; Unani formulation like *Qurs-e-Tabaasheer, KafooriLului (hectic fever), Qurs-e-TabaasheerMulaiyinin, Qurs-e-TabaasheerQabiz, Safoof-e-Tabaasheer and Jawarish-e-tabaasheer* [4].

Vanshlochans comprehended in both natural and synthetic way and sold without any solid official authentication system in Indian Market. For the first time Mr. Macie's preliminary studies established the physico-chemical data of various specimens of tabasheer by series of experiment with water, vegetable colors, with acids, with liquid alkali, dry alkaliesand other fluxes [5]. Klinowski J et al, also in augurated its analysis with modern analytical technique viz; X-ray diffraction (phase crystallinity), thermo-gravimetric analysis (TGA), solid state NMR, and X-ray fluorescence (elemental composition)[6].Recently studies were carried out on naturally available samples of vanslochan from Odhisa (India) for its microscopical, physico-chemical and heavy-metal analysis according to Indian Ayurvedic Pharmacopoeia [7].In contemporary times tabasheer is deliberately adulterated with burned bones, synthetic silica , and arrow root (substitute) in various formulation due to its improper procurement and unavailability. Generally Vanshlochana is formed only after twelve years of flowering period in hollow internodes of Bamboo [8]

Indian traditional medicine faces challenges in terms of regulatory approvals, safety and efficacy, standardization, and quality control.So there is a need of evidence based scientific studies for evaluation of the therapeutic efficacy and safety of Tabasheer. Present review article explains the pharmacological, analytical and clinical importance of the vanshlochan in Indian Ayurveda. Bambusha is an antique component in Indian traditional medicine; it is time to re-evaluate the various clinically well-documented indications inform ofreverse-pharmacology, molecular biology, proteomics, metabolomics, and networking pharmacology.

2. Chronological history of Bambusha

Bamboo manna originates from Sanskrit word – Tvak – kshira (Bark milk). In regional language of Indian subcontinent it is known as Sansk. - Vanshalochan, Venulavana, English-Bamboo manna,

Hindi-Banskarpur, Bengali-Bans-karpur, Bans, Marathi-Bansmitha, Telegu-Mullu Vedru, Unani-Tabasheer[9].From the ancient time tabasheer (bambusha) has gained interest as actual drug in eastern countries like India, China etc. China claimed it as "fossil teeth of china" and the belenities ("thunder bolts") due to its mode of occurrence. According to David Brewster claim, tabasheer is produced in particular those joint of bamboo which are in injured, unhealthy or inmalformed condition. It is also found in form of siliceous fluid (bambusha) inthose types of joints of stem where membrane lining cavities are destroyed or rent by disease [10]. The various studies related to tabasheer clarified concisely to reveal its controversy and original habitat [Table-1]. Alternatively the acquaintance about the tabasheer is basically familiarized in Western Europe by the Arabian Physician, but Patrick Russell (Vizagpattanam) criticized as a royalty of tabasheer as an Arabian[11]. Sir Joseph Banks was magnificently growing bamboo in a hot house at Islington – collected special tabasheer by method of splitting. Prof Andes collected tabasheer as American specimen, but their result is different from other specimens in composition[12]. Dr. Russell successfully collected liquid and solid form of tabasheer from different joints of bamboo. His results gave details about the character of the tabasheer i.e it is clear, transparent, colorless or greenish tint or white in colour, sometimes it is thicker and of a white colour and other times darker and having honey like consistency [13]. Prof. Edward Turner categories tabasheer as three types: chalky, translucent, and transparent also claimed Indian tabasheer consist entirely pure silica with minute quantity of lime and vegetable matter. D.W. Rostvan Tonningen studied Java specimen named tabasheer.

Table: 1.The chronological findings of the various author in context Vamshalochan (Tabasheer)

Author	Argument	Positive findings	References
Mr.Thiselton dyer (Attention name as Tabasheer)	Concentrate the interesting substance the respective community (Physicists, Botanist and mineralogist)	To categorize as vegetable kingdom and Mineral kingdom	[13]
Dr. David Brewster (Physicist) First Indian specimens which gave Dr. Kennedy	Curious product in vegetable kingdom (Tabasheer), Making thin section for examining under microscope.	The phosphorescent , opalescence property with white, opaque, thoroughly saturated with water, perfectly transparent and measure refractive index.[Colloid silica itself]	[13]

Sir Joseph Banks	Growing bamboo in a hot house at Islington – Collect special tabasheer in the method of splitting	A small pebble about the size of half a pea, externally dark brown or black colour with reddish – brown tint. So hard as to cut glass, crystalline structure in parts, Contain silica and iron	[13]
Prof Andes	South American (Pichincha) specimen gave MM. Fourcroy and Vauquelin.	A milk white colour, apparently crystalline, semitransparent and gelatinous. Ignition it became black, and emitted pungent fumes. 70% silica, 30% of potash, lime, water and organic matter.	[13]
Prof. Edward Turner	Indian tabasheer to consist almost entirely pure silica with minute quantity of lime and vegetable matter.	Tabasheer are categorized - chalky, translucent and transparent tabasheer. Specific gravity, Loss at 100^0C, red heat measured.	[13]
Guibourt	A theory of the mode of formation of tabasheer – Certain periods of its growth th bamboo needed less silica than at other times, and that when not needed, the silica was carried inwards and deposited in the interior.	Silica-96.94%, Potash and lime-0.13%, Water-2.93%, Organic matter-trace. Study different part of the bamboo- Ashes of the wood - 0.0612%.	[13]
D. W. Rost van Tonningen	Specimen tabasheer from Java named as "singkara" (island) It resembles like Indian Tabasheer.	Silica- 86.387, Iron oxide-0.424, Lime-0.244, Potash-4.806, Organic matter-0.507, water - 7.632 (%)	[13]

Rationality behind the Bambusha study:

The bamboo-manna is not a sugar, but a white, gritty body and salt like brittle between the teeth (Fig-1). According to Watts's (Dictionary of Chemistry) Bambusha is defined as "Hydrated silica, occurring in stony concretions from the joints of bamboo, it resembles hydrophane, and when thrown upon water does not sink till completely saturated[14]. For collecting the natural vanshlochan listening to the rattling sound of the bamboo is one of the acceptance criteria for presence and absence of tabasheer[15]. Small quantity of tabasheer is generally available in the

bottom and sides of the cavity of bamboo of the certain species like (*Bambusa arundinacea and Melocana bambusoides etc.*) Bambusha has greater abundance than others. Production of the tabasheer is greatly influenced by the soil, situation and season. The principal component of tabasheer i.e silica (amorphous, crystalline and colloidal form) is deliberately adulterated. On the contrary, requirement of dietary silicon and its mechanism of action is yet not clearly elucidated. Biogenic silica attributed with metal ions is an important part of biology. However there is still no evidence to support the idea of mechanism, silicon manifestation. The toxicity profile data is not available in approval of bamboo-manna. The ingredient (Tabasheer) containing various formulations hypothetically act as synergistically, antagonistically and supra-additively, bioavailabity enhancer, nano carrier.

Fig no -1, Marketed tabasheer (Bambusha) specimen

Experimental research of Bambusha in various environments

Bambush awas analysed in terms ofphysico-chemical, instrumental, pharmaceutical and standardization purpose which explained in table-2.

Account of fame chemical experiments ontabasheer (Bambusha) :

According to James Louis Macieand Co, seven sample were collected (Tabasheer), hydrated silica found within stems of some species of bamboo are used in medicine. From Various respective areas, sample are procured for studying tabasheer (Bambusha) which frequently treated with water, vegetable colours, fire, acids, liquid alkali, dry alkali, and other fluxes.

Table: 2. Research envisaged in context Bamsalochan

Research envisaged	Parameter performed	Positive findings	References
An account of fame chemical experiments on Tabasheer	Treated with water, vegetable colours, acids, liquid alkalies, dry alkalies and other fluxes.	Physico-chemical constant (like ash value, LOD, etc)	[5]
Structural studies of Tabasheer, an opal of plant origin	X-ray diffraction, and Fluorescence,Thermogravimetric analysis, Solid state NMR	$22^0\theta$ humpy peak, elemental composition,	[6]
Method of identification and standardization of Vamsalochan (Bamboo-manna)	Physico-chemical, Microscopic examination, Heavy metal analysis	Rosette crystal, silica-85.78%, Heavy metal is absent	[7]
Standardization of talisadichoorna and guti containing synthetic vanshlochan	X-ray diffraction, surface electron microscopy, Energy dispersive analysis, Thermo gravimetric analysis	Blurred peak at 20 degree, amorphous, crystalline, sodium, potassium, P, calcium,Fe and weight loss at 610 degree.	[8]

XRD, Thermo gravimetric analysis, XRF, Solid state NMR

Jacek Klinowski et al, appropriately utilized the modern analytical technique to proceed tabasheer physico-chemical fingerprinting profile. XRD pattern showed prominent broad peak in the region at about $22^{0}2\theta$ with amorphous nature. Thermogravimetric profile revealed gradual hydroxylation at $250^{0}C$ due to presence of bound water which established the "OH" presence.

X-ray fluorescence (XRF) showed that silicon is the major element along with other trace amount ofoxide form such as aluminium, calcium, magnesium, phosphorus. The solid state NMR established the presence of 4-coordinate (Tetrahedral) aluminiumin part of silicate network.1H NMR spectra successfully described two types of hydroxyl group and also explained proton-proton distance during dehydroxylation as a function of temperature. Intracellular biological silica generally completely aluminium free because of extracellular silica in bamboo is tendency to exclude aluminium from the plant cell.

Method of identification and standardization ofVanshlochan

Recently another study has been to standardize Vamsalochana with help of various physicochemical parameters like alcohol soluble extractive, water soluble extractive, total ash, acid insoluble ash were determined according to ayurvedic Pharmacopoeia of India (API). Microscopic examination revealed the presence of rosette crystals of calcium oxalate. Characteristic colours were obtained when the powder drug treated with different chemical reagents and solvents. Heavy metal analysis indicates the presence of silica as major component (85.78 %). Toxic metals like arsenic, cadmium, mercury and lead were absent.[7]

Future need in favor about bamboo-manna study:

The challenges are under regulatory status, the bambusha is lacking in assessment of the safety and efficacy in favor of national and international drug regulatory authorities. The silica is predominant in bambusha and is substantially used as substitute and adulterant substance but there is no clear valid document to fulfill the useof it. Although various traditional Ayurvedic formulation casts off one of the important ingredient in Ayurvedic Pharmacopoeia with-out any official valid proper documentation.

Conclusion:

A literature review has highlighted that Bambusha (Vamsalochan) exhibits important traditional clinical claim in context of expectorant, immunomodulator, various aliments. It also covered various physico-chemical, solid state analytical instrument the way of biogenic silica predominant along with trace element. Bambusha needs more authentic and

validated safety document to be used as medicine in traditional medicine. Also revalidated the efficacy claim in traditional claim with reverse-pharmacology, interdisclipinary way.

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Conflict of interest: The authors declare no conflict of interest.

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REVIEW ARTICLE

HYDROGELS AS NOVEL ALTERNATIVE FOR THE MANAGEMENT OF VARIOUS DISEASES OF CENTRAL NERVOUS SYSTEM

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Abstract

The word 'Hydrogel' includes 'Hydro' and 'Gel' indicating gel like property and water solubility. Hydrogel formation includes combining together of monomer units to form cross-linked structure which provides water insolubility. Hydrogel as a delivery system has gained wide acceptability due to its various applications. Based on the monomer used, hydrogel possess different properties and can be used for various diseases and disorders. Materials used for hydrogel formulation include Polyvinyl alcohol (PVA), Polyethylene oxide (PEO) and polysaccharides such as Chitosan, Cellulose, Agarose, Carragenan. Depending on the swelling behaviour, hydrogel can be used for different applications including controlled relase formulations The classification of hydrogel is based on the its physical, chemical, swelling, and source. Hydrogel based delivery system has application in effective management of various diseases of central nervous system like Alhzeimer; Parkinson's; Tumor of spinal cord; Down's syndrome and brain stroke. Different materials used in the formulation of the hydrogel provide different property thus making it suitable for management of different diseases and disorders besides central nervous system..

Keywords: Hydrogel, Cross linked polymers, Central nervous system, Controlled release.

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INTRODUCTION

A Hydrogel can be described as amalgamation monomer units linked to form a water soluble polymer which is further cross-linked to form an insoluble network. It is a water swollen network polymer. Hydrogels are already used in manufacturing of soft contact lenses and gelatin desserts. These cross-linked bonds may be covalent, ionic, hydrogen or simply physical entanglements having important role in determining physical characteristics of it. Amount of water present in the swollen state of hydrogel determines its properties and characteristics. Usually, higher the water content, the material has higher application potential. It can be utilised in various forms such as absorbent material, soft tissue renewal material, membrane for separation and moisture retention in soil. In addition to that, there has been substantial interest in altering the unique characteristics of hydrogel in controlled release technological area.

STRUCTURE AND PHYSICAL PROPERTIES OF HYDROGEL

Different water soluble monomers like hydrophilic vinyl-type monomers, sugars and amino acids which are obtained from natural resources are the fundamental building blocks of hydrogel. This monomer is then synthesised by a synthetic process. The crosslinking of the polymer generates hydrogel network might be carried out during polymerization or after the production of polymer. This final resulted monomer is characterised by the average distance between cross-links, which predominantly affects the properties of polymer.

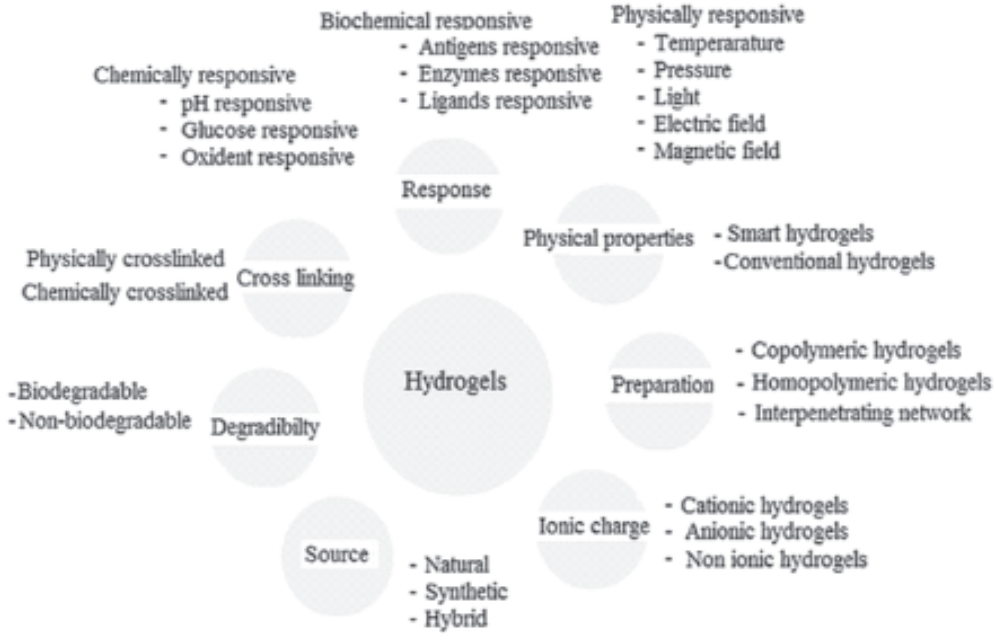

Figure 1: Classification of hydrogels

MATERIALS USED IN HYDROGEL

Different materials can be used in hydrogel manufacturing. This includes polyvinyl acetate (PVA), Polyethylene oxide (PEO), Poly N-vinyl pyyrolidone (PNVP), polysaccharides and polyphosphazenes [1]. Brief information about the materials is provided as below-

1) PVA (Polyvinyl alcohol)

Hydrolysis of polyvinyl acetate generates polyvinyl alcohol (PVA) which is characterised based on the basis of molecular weight and degree of hydrolysis. It is proved that if the PVA is fully hydrolysed then it possesses the best crystallizing properties like water solubility and then only can be dissolved in hot water. If it is 85-90% hydrolysed, then maximum cold water solubility can be achieved. The hydroxyl group is another better candidate which can give marvellous sites for cross-linking with such difunctional reagents. It possesses some extraordinary properties like crystalizing at low temperatures in solution which does not happen in normal crystallisation procedures of polymers. Based on research on mechanical properties of PVA gels, it has been found out by researchers that both stress at break and modulus had higher characteristics with the increasing number of freeze thaw cycles. Elongation at break was having opposite characteristics. Along with that, mechanical strength was increased with increment in degree of hydrolysis and molecular weight.

2) PEO (Poly Ethylene Oxide) based materials

Polyethylene glycol (PEG) and Polyethylene oxide (PEO) have almost same structure but difference in molecular weights. The former has low weight and the latter has high molecular weight. Similar to the PVA, PEO can also crystalize with keeping in its rather low temperature. Along with that, to have good hydrogel composition, chemical cross-linking is necessary which can be attained by di isocyanate compound. The aftermath product can be called a polyurethane with huge number of hydrophilic soft bank. A normal diffusion kinematics were observed from the release of caffeine and prostaglandin from diisocyanate cross-linked PEO by Graham but on the other hand a more complex release was found if the same was released from thee dry gels which ultimately showed a constant release rate. The equilibrium of the materials used to manufacture those ranges from 0.2 to 19 with respect to dry polymer. A very amusing material was prescribed by Stadler and Weber which helped linking PBD chains to PEO cross-links with the percentage varying from 3.2 to 16.2% wt and a large number of these products showed volume swelling more than 1.25 and some of the samples even reached the 1.5 scale. The results of transmission electron microscopy elaborated that materials had 2 continuous phases, the property by means of which they are capable enough of loading hydrophobic drugs into a water solvable system.

3) Poly N-Vinyl Pyrrolidone (PNVP) / PVP

Poly N-Vinyl Pyrrolidone (PNVP) / PVP which was initially used as a blood plasma extender has more hydrophilic gels polyhydroxy ethyl methacrylate (PHEMA) and swell to particular limit. The presence of water in the equilibrium position can be as high as 95% and the interaction parameter varies from 0.49 to 56 under the certain conditions. PNVP is often found to be copolymerized with less hydrophilic monomers because of its high hydrophobicity.

4) Polysaccharides

It includes the natural materials, which includes the bacterial fermentations and also taken from the plants. Generally the polysaccharides in the natural materials are used more than the other synthetic polysachharide materials. It also includes the functional groups like the acidic groups like the COOH in the gum arabic, Sulfate group in the caragenanas. The basic group contains the polymer like the chitosan, which is also frequently used.

1) Cellulose

It is one of the most frequently used polymer and forms the highly swellable gels. It is highly soluble in water and also compatible with it.

2) Agarose and Carrageenan

They are sulfated galactans. Both the polymers adopt linear and the helical structure. Thermally reversible gels are formed with the Aragose and the Carrageenan.

5) Polyphosphazene

It has very less application in the hydrogel applications. It forms rubbery type of the material and also cross-linked with the di and trivalent cations. It absorbs the high amount of water and generate the highly swollen gels.

HYDROGELS USED IN VAROIUS CNS DISEASE

INTRODUCTION

- Hydrogel in Down's Syndrome
- Hydrogel in Spinal Cord Disease
- Hydrogel in Alzheimer's Disease
- Hydrogel in Stroke
- Hydrogel in Tumour
- Hydrogel in Parkinson's Disease

Due to presence of blood brain barrier (BBB) and blood spinal cord barrier (BSCB), the transfer of molecules to the Central Nervous System is a confronting issue. There are some instances where direct cerebral spinal fluid injections into the extracellular fluid is also proposed and used in clinical and pre-clinical or experiments. The present research in the same field encompasses examining bio-inert as well as biocompatible polymers as base for controlled release of biologically active molecules inside the central nervous system for controlled drug delivery. It is

necessary to mention that there is an interim leak in the BBB and BSCB after injury or stroke to the spinal cord and brain, which permit molecules and cells to get into the Central Nervous System from the circulating blood than normal physiological conditions. It is still unforeseeable that up to which extent, this "leakiness" can be useful for cell and drug delivery. Polymeric hydrogels which have some automated characteristics with nervous tissue, can be placed in the intra spinal space so that it can balance homogeneous mechanical landscape with all adjacent soft central nervous system tissue. Hydrogels which are prepared from different synthetic and natural polymers are explored for its capability to deliver therapeutic activity directly into the spinal cord and brain.[2]

Fibrin-based hydrogels have been more used and progenitor/stem cells in combining with growth factors in rodent models of spinal cord injury and scar inhibiting enzyme chondroitinase ABC Injecting in- situ gelling form of agarose in combination with lipid microtubules filled with bioactive molecules after spinal cord injury have been used. Chitosan-based hydrogels incorporating micro particles or nanoparticles have been reported Refer Figure 2, Hydrogels in CNS. Molecules having bioactivity are delivered to the CNS using micro or nanoparticles of chitosan based hydrogels but chitosan-based biomaterials have widely been used for delivery through the nasal route.

This example of delivery is lucrative since the nasal passage has porous endothelial membrane.

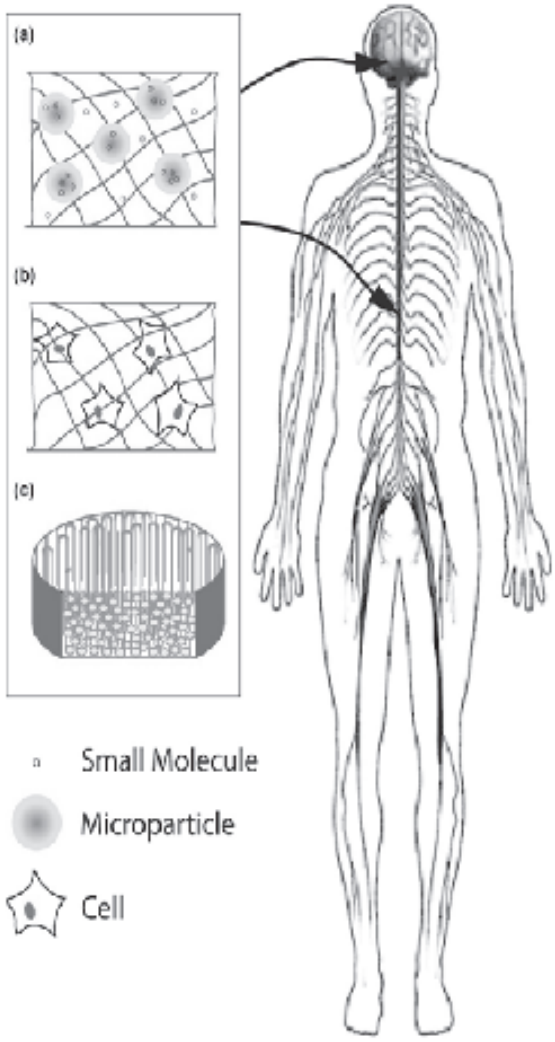

Figure 2: Hydrogel in CNS1

The figure represents the ways in which the hydrogels are developed for the therapies to repair the nervous system, here the microparticles used to create the highly tunable delivery platform for the small molecules in CNS, also hydrogels can also be used to deliver the cells in the CNS.

1) HYDROGEL IN SPINAL CORD

Spinal Cord Injury (SCI) is a condition for which still we got no cure. Individuals experiencing SCI have long lasting glitch of body parts and lessened personal satisfaction. Consistently as low as 2.3 to as high as 83 for each million occupants experience the ill effects of this. The auxiliary occasions after the essential damage comes about into increase in the multifaceted nature of the malady and because of that SCI treatment ends up harder. The present drug a spinal string injury is constrained. A few medications named the decompression of the rope and in the end the organization of calming medications and adjustment of the spine are accessible. Along these lines, the improvement of novel restorative systems focusing on this condition is urgent and exceptionally essential. Cell based treatments are a standout amongst the most as often as possible actualized and investigated among the diverse methodologies depicted previously. Fat tissue-inferred Stromal/Stem Cells (ASCs) and Olfactory Ensheathing Cells (OECs) have indicated better and confident outcomes. For instance, intra spinal transplantation of murine ASCs, which additionally actuated apparent picks up in engine execution, in a SCI creature show, one week after damage, advanced the assurance of stripped axons presumably by keeping oligodendrocytes' degeneration and by taking an interest in the recovery of the myelin sheath.[3]

The idea of the ASC's secretome chooses the useful results. ASC's secretome is the board of atoms discharged by these cells to the extracellular milieu. Truth be told, multitudinous confirmations demonstrated that ASC's secretome contains critical neuro administrative atoms, for example, Nerve Growth Factor (NGF), Glial cell line-inferred Neurotrophic Factor (GDNF), Brain-determined Neurotrophic Factor (BDNF), Insulin-like Growth Factor 1 (IGF1), Vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF), essential Fibroblast Growth Factor (bFGF), Transforming Growth Factor Beta 1 (TGF-b1). Notwithstanding that, it is discovered that the particles emitted by ASCs have the ability to tweak and influence the reaction of the safe framework. Then again, OECs are primarily separated by partaking in the development and direction of essential olfactory neurons. Their aggregate root with Schwann cells may expand some coordinating qualities appeared between these two cell composes, specifically the limit of OECs to encompass olfactory axons, shape fascicular forms and orchestrate fringe like myelin. The potential OEC transplantation as a treatment for CNS harm has just been investigated in vivo. Most examinations utilizing these cells demonstrated the upgrades in conduct comes about. For instance, murine OECs could remyelinate axons in spinal string harmed rats which extreme prompt practical improvement of electric conduction in already demyelinated axons.

In this manner, it is considered that OECs can make a tolerant feel with the goal that axonal can be recovered, in the typically unfriendly milieu of the harmed CNS. For every one of these grounds, autologous transplantation of OECs in SCI patients has just been completed. There was a one clinical trialing which it was seen that autologous OECs are sheltered following three years post-transplantation. For the most part since they are effectively available, ASCs (which can be gotten in extensive amounts from lipoaspirates) and OECs (which can be securely disconnected from nasal biopsies) introduce themselves as promising contender for SCI cell treatment and can be connected in an autologous way with evading moral concerns and the requirement for immunosuppression. We picture exploiting the useful qualities of every cell compose at the same time by consolidating both.

2) HYDROGEL IN ALZHEIMER

There are various serious causes generated with the Alzheimer disease, which includes the various symptom like memory problem, thinking problem, behavioral problem, so it's brain disorder. In the United States the Alzheimer's disease is ranked at the 6th position. It's important to in the Alzheimer disease to relieve the neurodegenerative progression. Early diagnosis of the Alzheimer clinical analysis done of the cerebrospinal fluid and protein such as the beta amyloid peptide. Positron emission tomography is done for the AD. In the clinical examination there is the high cost for the PET and also the cerebrospinal fluid. MicroRNA is important so the attention is paid to it in AD, because of it modulate translation of messenger RNAs to protein by cleaving or destabilizing the mRNAs. Extracellular vesicles are enriched with the

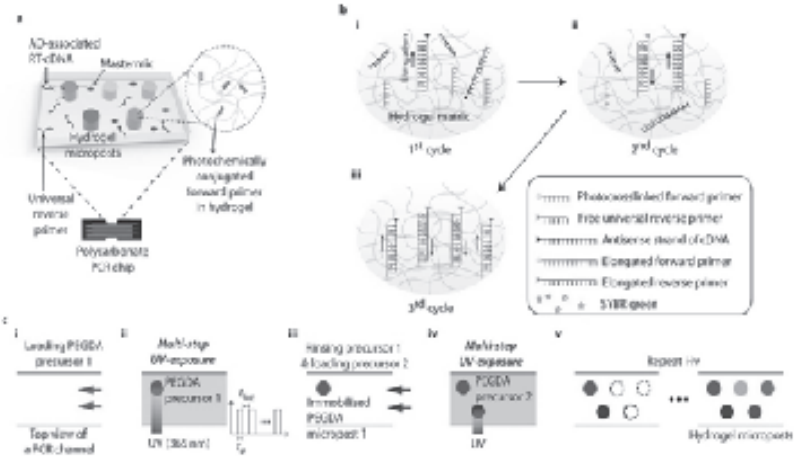

Figure 3: Hydrogel in Alzheimer's disease

Here, the diagram represents the (a) Schematic illustration of hydrogel microposts immobilized in a polycarbonate (PC) PCR chip in the presence of universal reverse primers and reverse transcribed (RT)-cDNAs associated with Alzheimer's disease (b) Schematic illustration depicting the principle of PCR confined within a hydrogel matrix. (c) Fabrication process to immobilize polyethylene glycol (PEGDA) microposts in the PCR chip.

micro RNAS by recent research which are the biomarker for the AD. Range within 10-fold seen between AD patients and healthy controls.[4]

3) HYDROGEL IN 3D TUMOR ENGINEERING

Now a days Hydrogels are used more also for the tissue engineering and also for the 3D culture cells because they possess very high biocompatibility and they have distinct characteristics which matches with that of living tissue which gives a compatible ambience for cells and make their response matching with that observed into the living organism. Changes in the hydrogel can be done with the help of the chemical modification to do the same thing as done by living tissues so resulting into more biocompatibility and enhance their in vivo working. Various kinds of polymers have been used to form the different hydrogel. Poly lactic acid (PLA) like synthetic materials, polyethylene oxide(PEO) , polyethylene glycol (PEG), polyvinyl alcohol (PVA), agarose and alginate, organosilicon-based nanocomposites and chitosan have been investigated so that they can be used with various kind of tissues. [4]

Likewise pores of these hydrogels and nano-sized strands emulate the structure of living tissues in-vivo which at last gives a domain that can procedure in-vivo cell–cell and cell-platform connections. Alongside that, fibre crosslinking by which hydrogels frame from these peptides don't require any synthetic added substances and UV light or warmth treatment which may prompt lower cell biocompatibility, not at all like the circumstance with other biopolymer-based hydrogels. Toward the end, infusion can create these peptide hydrogels which make them empower them to embody cells for 3D societies. Till now, different sorts of self-amassing peptide hydrogels have been used for biomedical applications going from hydrogels for tissue designing to nano-vehicles for hostile to malignancy medication and si-RNA conveyance.

4) HYDROGEL IN BRAIN AFTER STROKE

To substitute the lost tissue, ex vivo generation of engineered organs by means of classical tissue engineering technique can be implanted. As it requires the invasive implantation of the tissue construct, this approach is not well organized and proper for brain repair. [5] Another technique is In situ tissue regeneration which aims to completely bypass the ex vivo generation of the engineered organ. It does so by implanting a scaffold directly at the site of injury just to stimulate endogenous tissue repair by mean of local or transplanted progenitors. Although earlier the materials for brain repair used implantable materials, contemporary tries have been focused on engineered injectable hydrogels. [5]

The hydrogels can be designed to match the mechanical properties of the normal

brain by modulating the crosslinking density and to serve as local drug delivery depots. The crosslinking point and network mesh size depends upon the polymer chain length and its tendency to percolate and these are factors by which the characteristics if it can be modulated, which at the end and ultimately modulates nutrient diffusion and cell motility. [6]

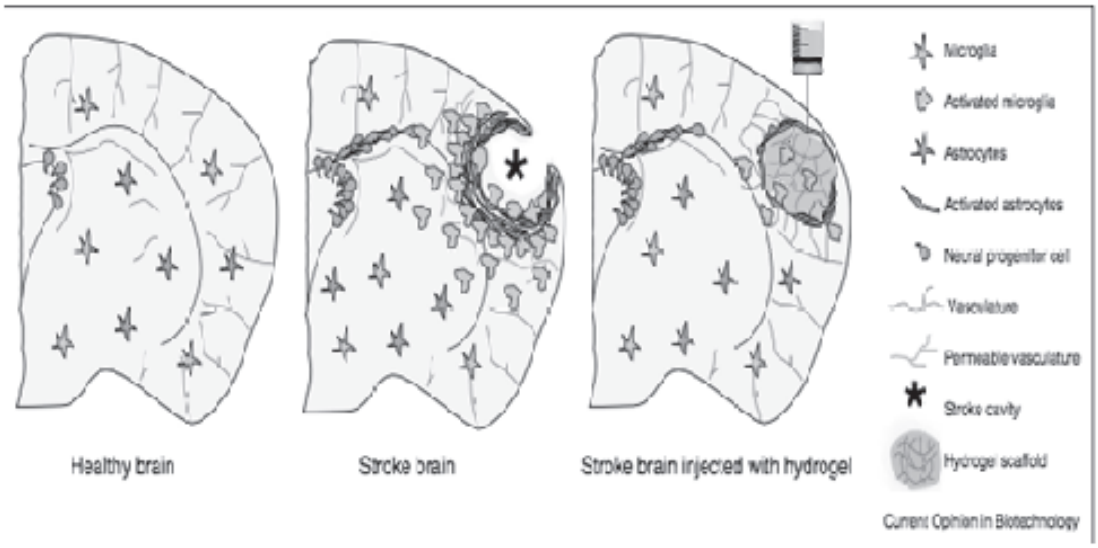

Figure No. 4: Hydrogel in Brain Stroke

Diagram represents the coronal brain section and the major physio-pathological events occurring after an ischemic stroke.

5) THE HYDROGEL IN STEM CELLS

To treat different diseases, adult stem cells, which are capable enough of being self-renewable, are good option to go with as they provide therapeutic applications in a nice way. To treat the neurodegenerative diseases (Parkinson's disease, stroke, Alzheimer's disease, and spinal cord injury) recently, neural stem cell, which are technically difficult when the matter comes to isolation and characterization thus leading them to be less used, therapy has been taken into consideration as a whole new approach. Adult stem cells, in addition to that, possess an inherent capacity to differentiate into many cell types such as fibroblasts, osteoblasts, adipocytes, yocytes, and neurons, and due to this property, these adult stem cells could act as an unlimited source of stem cells which is highly useful in treating neurodegenerative diseases.[7]

Among the all viable sources of adult stem cells like bone marrow, adipose tissue, muscle and human umbilical cord, considerable attention has been centered on skeletal muscle because it is a convenient and abundant source of adult stem cells due to its considerable mass in

the body. If the strategies to isolate and characterise skeletal muscle from muscle-derived stem cell is developed then it will pave the way towards generation of unlimited stem cells. This could be achieved by MDSC because it can differentiate into neurons and glial cells, e.g. oligodendrocytes and astrocytes. To treat epilepsy and bipolar disorder, Valproic acid (VA), which can even act as a pleiotropic histone deacetylase (HDAC) inhibitor which helps contributing into differentiation in process, is a proliferatively used because it is an anticonvulsant and mood stabilizing drug but the previous results are not that much convincing as they showed low cell survival. However, the survival issue of adult stem cells can be eliminated if a number of 3 dimensional scaffolds with geometric or topographical features are provided. The hydrogel could be a 3-dimensional scaffold as it can be fabricated even with scaffold geometry's complexity's existence. Chitosan, which is abundant and natural, is another attractive candidate for the same purpose. Hydrogels which are based on chitosan are kown to have some better features like biodegradability, biocompatibility and non-immunogenic which lead it to the best candidate for scaffold tissue engineering processes such as cell encapsulation and cell culture. Recent studies have shown that a mixture of chitosan and glycerol phosphate disodium salt (GP) can form a hydrogel in situ at body temperature. However still, no scientific studies have tested the differentiation of MDSCs in the presence of VA on chitosan-based hydrogels, yet to be completed. The ultimate and final aim of this work was to treat the neurodegenerative diseases through tissue engineering in rat MDSCs in the presence of VA on chitosan-based hydrogels.

6) LYMPHOMA:

Methotrexate (MTX) is crucial therapeutic to treat number of malfunctionalities of central nervous system, but has a slight fall that it passes poorly through blood-brain barrier. Fortunately, muco-adhesive chitosan based nano-formulation if accompanied by intranasal Administration could be a key to this hurdle. **Methods:** Nanogel having MTX was differentiated on the basis of morphology, drug loading, particle size, drug release behaviour and zeta potential after it was prepared by ionic gelation method. The resulted were compared with the eight maths models such that a particular phenomenon could be understood. The the solution with deionized water was administered into the nasal cavity of rats and after 15, 30, 60, 240 minutes, the plasma and brains were analysed for MTX quantity. **Results:** Particle size = 100 nm, zeta potential = 18.65 ± 1.77 mV, loading efficiency = 65.46 ± 7.66, loading capacity = 3.02 ± 0.34. The release phenomenon was found to be complying Swelling and Fickian diffusion. Results of *in vivo* studies : Drug Targeting Efficiency = 424.88%, Direct Transport Percentage for Nanogel (test)

and Drug free solution (Control) are 76.46% and 34842.15% and 99.71% respectively. **Conclusion:** More amount of MTX was generated by Nanogel in brain but not in plasma if compared with free drug solution. Further, it was found that Intranasal Administration increases the brain concentration of MTX.[8]

CONCLUSION AND FUTURE PERSPECTIVE

The research on hydrogels have witnessed a tremendous enhancement for therapeutic delivery, producing new hydrogels which are application oriented like multi responsive hydrogels and tandem gelling hydrogels, along with simultaneously achieving unprecedented things by means of exceptional qualities like facile administration in the same field. The advancement move towards Top notch technological development in which the world is being engulfed is now leading to the 3D printed hydrogels. If combined with biologically active agents, then lucid sway over cellular behaviour could be obtained in the field of tissue engineering. The nano-cells having rapid response and high adaptability to ambience is another lucrative candidate for the same field responded with intracellular clues, making bio-orthogonal crosslinking superior to classical linking because of its fast reaction kinetics. Hydrogel systems has some basic requirements like formation of in situ is a basic requirement, use of bio-orthogonal reactions to fasten the environmental response and drug delivery in specified quantity, all which could be easily obtained by modulation of release of hydrogels administration leading to greater control in drug concentration.

There are some critical issues related to manufacturing and stability which need to be resolved. However, these new advancements in therapeutic hydrogels have potential to develop safe, efficient and scalable drug delivery which could to be commercialized.

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