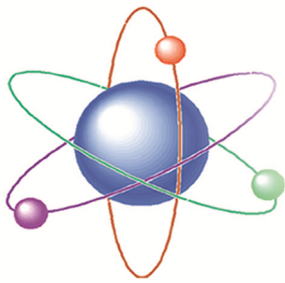


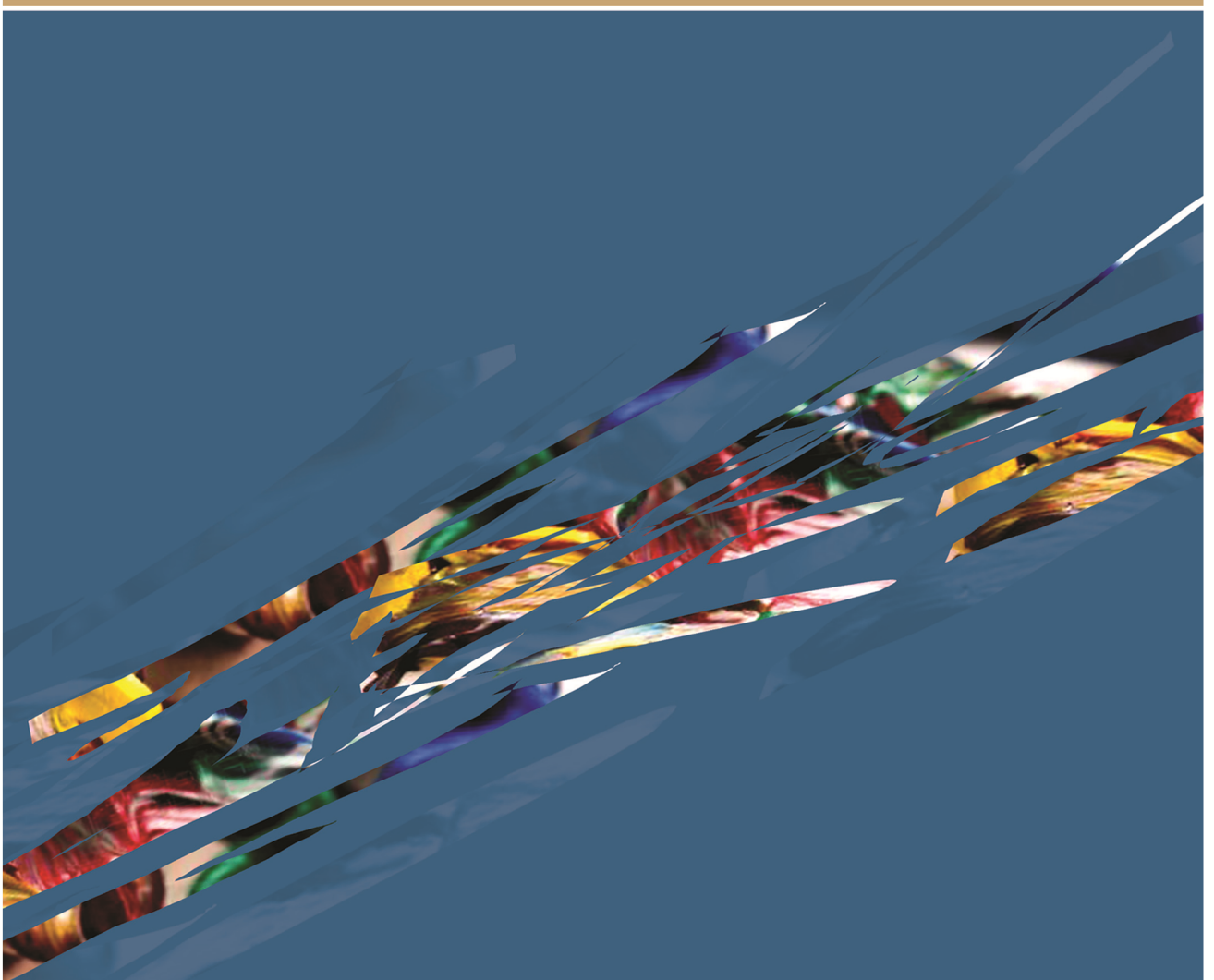
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Editorial

Integrating Indian Knowledge Systems into Higher Education

In recent years, the Indian Knowledge System (IKS) has emerged as a critical component of higher education reform in India. Far from being merely a cultural revival, the integration of IKS offers a framework for holistic, interdisciplinary, and sustainability-oriented education. The National Education Policy (NEP) 2020 emphasizes systematic inclusion of India's classical knowledge traditions, creating opportunities to bridge historical intellectual heritage with contemporary academic demands (National Education Policy, 2020; Sharma, 2022).

IKS encompasses a broad spectrum of disciplines, including mathematics, astronomy, medicine, philosophy, linguistics, and governance, representing centuries of sophisticated scholarship (Mehta & Singh, 2021). Historically, institutions such as Takshashila and Nalanda exemplified global centers of learning, and their pedagogical principles—mentorship, experiential learning, and ethical education—remain relevant today (Joshi & Bansal, 2023). Embedding these traditions within modern curricula can nurture students who are academically competent, socially responsible, and culturally grounded.

A compelling justification for integrating IKS lies in its alignment with sustainable development principles. Traditional practices in agriculture, water management, and ecological conservation reflect deep insights into environmental stewardship, ethical living, and community well-being (Mehta & Singh, 2021). Concepts such as *prakriti* (nature), *dharma* (ethical duty), and *lokasangraha* (collective welfare) can inform contemporary education, particularly in environmental sciences, public policy, and management programs.

Pedagogically, IKS fosters critical thinking, problem-solving, and interdisciplinary inquiry. Experiential learning, mentorship models, and value-based education embedded within traditional systems resonate with modern student-centered, project-based, and holistic learning approaches (Joshi & Bansal, 2023). Classical scientific texts also demonstrate advanced understanding in mathematics, astronomy, and medicine, challenging the notion that indigenous knowledge is incompatible with empirical science (Sharma, 2022).

However, meaningful integration requires careful design and academic rigor. Without structured curriculum frameworks, trained faculty, and standardized research methodologies, IKS risks superficial adoption or misrepresentation. It is essential to balance cultural revival with scholarly critique, ensuring that IKS complements rather than competes with contemporary global knowledge systems (Joshi & Bansal, 2023).

The potential for research-oriented initiatives within higher education is substantial. Interdisciplinary programs combining IKS with modern disciplines can enhance innovation in health sciences, environmental management, and technology. Institutional support for IKS-focused research, digital archiving of manuscripts, and collaborative projects can facilitate critical scholarship, increasing global relevance and visibility of Indian knowledge traditions (Mehta & Singh, 2021; Sharma, 2022).

In conclusion, integrating the Indian Knowledge System into higher education represents a transformative opportunity. By fostering interdisciplinary learning, ecological consciousness, and cultural rootedness, IKS can

contribute to producing graduates equipped to navigate global challenges responsibly and ethically. Institutionalizing these systems with academic rigor, critical engagement, and sustained policy support will create an educational model that honors India's intellectual heritage while addressing contemporary societal needs.

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Plant Metabolomics: A Cutting-Edge Approach

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Abstract:

Metabolomics is a dynamic and multidisciplinary area of research focusing on the identification and quantification of metabolites, small molecules produced within cells, biofluids, tissues or organisms as a result of metabolic processes. Through the analyses of metabolites, such as amino acids, sugars, and lipids, researchers can understand the valuable insights into an organism's metabolic state and its response to environmental and genetic changes. It is a rapidly advancing area of scientific research that combines systems biology, biochemistry and analytical chemistry to find out the intricate inter-relationship between metabolites and their surroundings. The rise of metabolomics as a field has been driven by the advances in analytical technology. Various tools like gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectroscopy, high-resolution mass spectrometry (HRMS), and matrix-assisted laser desorption/ionization (MALDI) have unlocked new ways to study and understand the metabolites. These advanced techniques allow scientists to identify and quantify from extremely small molecules to large molecules in unprecedented detail, making it possible to explore the metabolic processes of living organisms more profoundly than ever before. In the case of plant metabolomics, the focus is specifically on the metabolites found in plant cells and tissues. By analyzing these compounds, researchers can get a deeper understanding of how plant metabolites function, influencing growth, development, and the ability to respond to stresses. It helps to identify the important metabolites tied to specific traits, key metabolic pathways, and explains how plants can adapt to environmental and genetic changes. In this review, we have briefly discussed the untargeted metabolomics, its workflow and applications with emphasis on plants.

Keywords: Plant metabolomics; untargeted metabolomics; mass spectrometry; data analysis; metabolites

INTRODUCTION

Metabolomics is a dynamic and multidisciplinary area of research focusing on the identification and quantification of metabolites, small molecules produced within cells, biofluids, tissues or organisms as a result of metabolic processes (Clish 2015a). Through the analyses of

metabolites, such as amino acids, sugars, and lipids, researchers can understand the valuable insights into an organism's metabolic state and its response to environmental and genetic changes (Rinschen et al. 2019). It is rapidly advancing area of scientific research that combines systems biology, biochemistry and analytical chemistry to

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find out the intricate inter-relationship between metabolites and their surrounding (Piazza et al. 2018; Manickam et al. 2023). The rise of metabolomics as a field has been driven by the advances in analytical technology. Various tools like gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectroscopy, high-resolution mass spectrometry (HRMS), and matrix-assisted laser desorption/ionization (MALDI) have unlocked new ways to study and understand the metabolites. These advanced techniques allow scientists to identify and quantify from extremely small molecules to large molecules in unprecedented detail, making it possible to explore the metabolic processes of living organisms more profoundly than ever before (Clish 2015b). This advanced approach allows scientists to detect and measure metabolites in complex samples such as tissue extracts, blood, serum, and urine etc. In the case of plant metabolomics, the focus is specifically on the metabolites found in plant cells and tissues. By analyzing these compounds, researchers can get a deeper understanding of how plant metabolites function, influencing growth, development, and the ability to respond to stresses. It helps to identify the important metabolites tied to specific traits, key metabolic pathways, and explains how plants can adapt to environmental and genetic changes (Manickam et al. 2023). Moreover, metabolomics in agricultural science investigates the metabolic pathways that facilitate crop growth and development. This also enables the identification of metabolites enhancing crop nutritional value and development of nutrient-enriched crop varieties (Ibarra-Estrada et al. 2016). Depending on the research approaches, metabolomics can be divided into two primary categories: targeted and untargeted (Lelli et al., 2021). Targeted metabolomics mainly involves in precise identification and quantification of a predefined set of well-characterized metabolites, typically those associated with a specific metabolic pathway or stress response mechanism (Roberts et

al. 2012a). This utilizes the already established internal standards for semi-quantitative or quantitative analysis. In contrast, untargeted metabolomics deals with a discovery-driven approach, looking for comprehensively identification and quantification of all metabolites present in a biological sample, regardless of their known or unknown identity, function, or biological significance (Schrimpe-Rutledge et al., 2016; Snart et al., 2015). This produces vast datasets, requiring advanced statistical tools like multivariate analysis to extract meaningful insights and simplify the data (Chen *et al.* 2022). In this article, we have briefly discussed the workflow of untargeted plant metabolomic analysis, from sample preparation to data analysis. The protocols for targeted metabolomics have been detailed well by Roberts and team (Roberts et al. 2012b).

1. Sample preparation

Sample preparation is critical in metabolomic studies, significantly impacting result reliability. Small variations in sample collection, metabolite extraction, or storage can compromise metabolite stability, leading to substantial changes in the observed results. To mitigate this, metabolomics samples must be collected uniformly and rapidly, considering the rapid enzymatic turnover rate of the metabolome (Salem et al. 2020). Plants parts like leaf, root, stems etc. can be used for metabolite extraction. Rapid freezing (flash-freezing) methods using liquid nitrogen (or dry ice) is strongly recommended for preserving fresh samples during harvesting to stop enzyme-induced metabolic changes. This immediate freezing step effectively halts metabolic activity, ensuring the integrity of the sample for subsequent metabolomics analysis (Sumner et al. 2007). The preparation of plant samples for metabolomics analysis typically encompasses four distinct phases: harvesting, drying (optional, fresh samples can also be used for extraction), extraction (if required), and sample preparation for analysis. Minor changes in sample

preparation is allowed depending on the analytical method and experimental design (Kim and Verpoorte 2010). Change in environmental condition induces variation in metabolome composition of the samples to be studied. Therefore, samples are generally grown under controlled environmental condition or collected from the same environment at the same time and condition to minimize the variation. Post-harvest, immediate freezing is crucial to halt enzymatic reactions triggered by handling and wounding. The most effective method is to flash-freeze the samples in liquid nitrogen to preserve metabolite integrity and quenching (Sumner et al. 2007). In some cases, such as NMR analysis, drying is essential before extraction of the plant metabolome. Flash-frozen samples can be stored in -80°C for a short period of time. Before metabolite extraction, samples can be lysed, lyophilized or homogenized according to the experimental requirement (Salem et al. 2020).

2. Metabolite extraction

Effective metabolite extraction is crucial for successful metabolomics experiments. For untargeted metabolomics, optimal extraction methods are vital to release a diverse spectrum of metabolites, ensuring comprehensive analysis and accurate representation of the sample's metabolome (Roberts et al. 2012b). An appropriate extraction solvent is chosen based on polarity, which governs metabolite solubility ('like-dissolves-like' principle); selectivity, targeting specific metabolite classes and toxicity and inertness, ensuring safe and unbiased extraction. For novel, exploratory studies, a series of solvents may require to be tested for ensuring maximum metabolite extraction. For LC/MS based practices, methanol or acetonitrile acidified with 0.1% formic acid is widely used for untargeted metabolite extraction. For GC/MS, however, volatile organic solvents such as ethyl acetate, dichloromethane, toluene and hexane etc. are used (Gaylor et al. 2018). Derivatization, a chemical modification that enhances the separation and

sensitivity of detection of analytes is mandatory in GC/MS based practices. A set of biological replicates and a pooled QC stock sample prepared by pooling a specific volume from each sample of the experiment is highly recommended. This increases the reproducibility of the experiment and helps in signal normalization and identification and annotation of unknown metabolite (Souza and Patti 2021). Plant tissues such as leaves, stems or roots are homogenized well using liquid nitrogen, vortexed, ultrasonicated and centrifuged in the extraction solvent and the supernatant is collected for analysis.

3. Sample analysis

Although untargeted metabolomics aims to identify the global metabolome in the studied sample, it faces limitations due to the diverse chemical properties of metabolites. Experimental conditions, such as solvents and instrumentation, affect which metabolites are detectable. In general, methods that are suitable for only a specific class or few are avoided as this decreases the global metabolome coverage of the sample. Liquid Chromatography/Mass Spectrometry (LC/MS) is the gold standard for untargeted metabolomic study due to its capability to detect numerous metabolites and highly sensitive nature (Souza and Patti 2021). A general untargeted plant metabolomic analysis starts with extracting the frozen plant tissue (e.g. 100 mg) in methanol/water (80:20, v/v) using 10-minute ultrasonication and 13,000 rpm centrifugation for 10 minutes. The resulting supernatant is evaporated and reconstituted in LC-MS grade solvent (50% acetonitrile, 0.1% formic acid). For LC-MS-based metabolomics analysis, pre-filtering of the samples using 0.22 µm nylon syringe filters is crucial for removing contaminants, preventing column clogging and to enhance the efficacy of the experiment. LC-MS analysis generally utilizes a reverse-phase C18 column with a 0-20 minute gradient elution (5-100% acetonitrile) at 0.3 mL/min. Electrospray ionization mass spectrometry (ESI-MS) within a scanning range of 50-1000 m/z

in both positive and negative polarities is suggested (Want et al. 2013).

4. Processing of raw files

In untargeted plant metabolomics, raw LC-MS files are processed through a series of steps to extract meaningful information. Initially, raw files are imported into specialized software (e.g., Progenesis QI, MetaboAnalyst, XCMS, MZmine) for peak detection, alignment, and feature extraction. Noise, isotopes, and adducts are removed through data filtering, followed by normalization (e.g., total sum normalization) to correct for variability. A typical MS data processing workflow consists of importing the raw data file, mass detection, smoothing/filtering (optional), peak picking, deisotoping, alignment, gap filling, and annotation. Metabolite identification is performed using databases (e.g., KEGG, PlantCyc). Finally, pathway analysis is conducted to understand the overall metabolic network. Statistical analysis (e.g., PCA, ANOVA) is then applied to identify significant metabolites. MetaboAnalyst (Pang et al. 2024), MZmine (Schmid et al. 2023), XCMS (Tautenhahn et al. 2012) are widely used user-friendly, open-source platforms that enable investigators to pre-process, analyse and visualize large metabolomic datasets easily. A graphic representation of the data-processing workflow is given in **Figure 1**. All the tools follow almost the same operating protocol, from raw data processing to statistical analysis. Here, we have discussed briefly the steps followed in MZmine3.3.0. This tool supports .mzML, .mzXML, .imzML, .netCDF, and .aird file formats. Raw files can be converted to any of these formats utilizing msConvert, a command-line utility for converting mass-spectrometry file format. msConvert enhances the quality of mass spectrometry data through peak picking, which extracts prominent peaks and minimizes noise and irrelevant information. This refined data facilitates accurate identification and quantification of target compounds by downstream analysis tools. Following conversion to .mzXML files, the data is

imported into MZmine 3.3.0 software via the "Raw Data Import" tool under the "Raw Data Methods" tab. Once all files are selected, mass detection is performed by navigating to "Raw Data Methods" and accessing "Feature Detection" and then "Mass Detection", enabling comprehensive analysis.

In MZmine 3.3.0, the centroid mass detector processes raw mass spectrometry data to identify and extract individual peaks. This centroiding process converts complex mass spectra into discrete peaks representing single compounds or ions. For feature detection and chromatogram construction, the ADAP chromatogram builder module identifies target compounds' m/z values based on user-defined lists. It then extracts corresponding intensity values and constructs chromatograms. Adjustments can be made as needed to suit experimental requirements. Chromatogram resolution is achieved through the Local Minimum Resolver, accessible via Feature Detection > Chromatogram Resolving > Local Minimum Resolver. This critical feature-resolving step separates overlapping and co-eluting peaks, ensuring accurate data pre-processing. In this process, parameters like chromatographic threshold, minimum absolute height, peak duration range etc. are to be adjusted based on the experiment. To eliminate the repetitive features, for example, the ones generated due to the presence of isotopes, isotope filters is applied which removes the possible isotope signals. In mass spectrometry, ionization of a compound can generate multiple peaks representing different isotopes. For instance, a compound with one carbon and two nitrogen atoms (C1N2) may produce peaks at m/z 42, 43, and 44, corresponding to carbon-12, carbon-13, nitrogen-14, and nitrogen-15 isotopes. The Isotope Pattern Finder tool, accessible via "Feature List Methods" > "Isotopes" > "Isotope Pattern Finder", identifies these isotopic peaks to confirm compound identity. Following isotopic analysis, the Join Aligner module aligns corresponding features

across samples based on mass, retention time, and user-defined tolerance ranges. This module is accessed through "Feature List Methods" > "Alignment" > "Join Aligner". To optimize the results, various parameters within the "Join Aligner" module can be adjusted according to the research objectives.

To improve the quality of the raw feature data, a "Feature List Filtering" step is used to remove low-quality or noisy features based on set criteria, ensuring that the dataset remains reliable. Further, the "Gap Filling" step is applied to address missing data, which could result from instrument errors, sample handling problems, or other limitations. This is an important step which helps in making the dataset more complete, enabling better statistical analysis and easier identification of unknown metabolites. Once this process is completed, the cleaned-up feature list is matched against the various databases like, KEGG database for molecular annotations. Lastly, the annotated results can be exported for further analysis and visualization, providing a more complete picture of the metabolomic findings.

5. Statistical Analysis

Analyzing metabolomic data involves different statistical techniques based on the type of experiment being conducted. Researchers often use both the univariate and multivariate analyses. Univariate analysis provides a straightforward look at the data, making it easier to identify important conditions. For comparing two groups, methods such as t-tests, fold change analysis, and volcano plots are commonly used. When looking at multiple groups, one-way ANOVA is usually the go-to method, and researchers often follow up with post hoc tests and correlation analysis to dig deeper into the relationships between the variables (Cambiaghi et al. 2017). With many metabolites present in each sample, the risk of false positives may increase during multiple testing. To avoid this, researchers mostly use the correction methods like Bonferroni adjustment and Benjamini-Hochberg's False Discovery Rate (FDR) to refine p-values and minimize false positives.

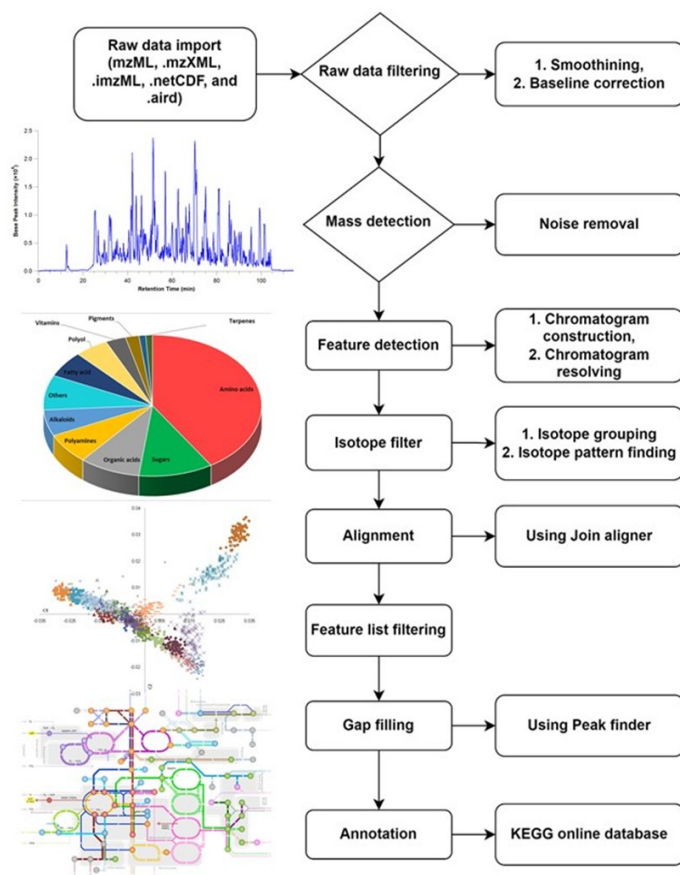
Multivariate analyses play a crucial role in making sense of complex data by looking at several statistical variables at the same time. This approach gives researchers a clearer picture of the underlying patterns. These methods are particularly helpful when we are dealing with exploratory datasets, as they simplify large amounts of information into few and to more significant variables. The toolbox for multivariate analysis includes techniques like multiple regression, principal component analysis (PCA), factor analysis, ANOVA, and partial least squares discriminant analysis (PLS). Moreover, machine learning methods such as support vector machines (SVM) and random forests are also part of this toolkit. Cluster analysis adds another dimension by revealing hidden patterns and groupings within the data. By utilizing these advanced statistical techniques, researchers can uncover valuable insights from intricate datasets, paving the way for new discoveries and innovations (Chen et al. 2022). In untargeted plant metabolomics, PCA is a key tool for handling complex datasets. This powerful statistical method helps ensure data quality and consistency by identifying patterns, trends, outliers, and anomalies, which in turn validates both instrument performance and sample reliability. It also reduces its dimensionality, making it easier to visualize relationships between samples and metabolites. This allows researchers to uncover correlated metabolic networks and understanding the deeper scientific explorations. (Chen et al. 2022).

6. Applications of untargeted plant metabolomics

As plant science continues to evolve, metabolomics has emerged as a powerful tool that offers profound insights into the complex cellular processes that underpin plant biology. The wide variety of metabolites produced by plants is essential for numerous functions, including regulating growth and development, producing vibrant pigments, creating aromatic compounds, and enhancing resilience against environmental stressors. Gaining a deeper understanding of these metabolites is crucial for unraveling how factors

like the environment and genetic traits influence plant physiology and biochemistry. By identifying and quantifying these metabolites, researchers can interpret the intricate language of plant metabolism, revealing how plants respond to stress, adapt to changing conditions, and improve their growth and productivity. This knowledge is vital for developing crops that are more resilient, sustainable, and high-yielding, ultimately supporting global food security and promoting environmental sustainability (Bozza *et al.* 2024). Scientists are exploring to understand how plants respond to different environmental stressors through metabolically, and aiming to understand what makes crops resilient. Exposing to challenges

like extreme weather or pests, plants activate intricate defense strategies, adjusting their internal chemistry and producing special protective compounds (metabolites). Understanding these natural adaptations became the key to developing crops which can better endure climate change and resist to diseases (Litvinov *et al.* 2021). Untargeted metabolomics may also be utilized for rapid identification and quantification of phytonutrients (known as phytochemicals or antioxidants) (Girija *et al.* 2021). In recent years, it is extensively used to investigate biochemical changes occurring before and after the harvesting of various crops, including vegetables, fruits, grains and ornamental plants.



7. Conclusion

Plant metabolomics has emerged as a powerful tool of research in recent years because it provides a comprehensive view of the metabolic status of plant physiology and metabolism. However, the metabolomics analysis presents several challenges such as the complexity and diversity of the metabolome, a dynamic range of metabolite concentrations and variability in sample preparation. The cost of procuring the analytical instruments and high purity chemicals pose another challenge for the budding researchers or scientists. Knowledge on computational analysis and statistics is also an indispensable prerequisite. However, this has been substantially reduced through the development of several easy-to-use, open-source, web-based or installable tools in the recent years. To be completely comprehended, untargeted metabolomic data must be combined and processed with other omic data. Nowadays, the most popular method is to perform integration analysis to extract structural and dynamic changes in the underlying biological network of interest while concurrently monitoring the levels of transcripts, proteins, and metabolites. In future, advances in technology and analytics will propel plant metabolomics forward, deepening our understanding of plant systems and unlocking innovative, sustainable solutions.

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Nuclear medicine and radiopharmaceuticals for treatment of diseases: An overview

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ABSTRACT

The increase of chronic diseases worldwide led to the development of various newer medicines and diagnostic techniques. Over the past decades with the advancement in science and technology health sector has witnessed significant advancement in identification and treatment of critical diseases like cancer, cardiovascular diseases etc. One of the important processes is the use of radioactive compounds. The clinical use of trace number of radionuclides is referred to as nuclear medicine. It is used for the diagnosis, therapy and monitoring the diseases. The radionuclides used in treatment or diagnosis process are also termed as radiopharmaceuticals. The radioisotopes decay radiations like, alpha, beta or gamma radiation. The alpha and beta emitted radio isotopes are used for the treatment of cancer where as isotopes that decay gamma radiation are used in combination with cameras and scanners to take the images of the processes and structure in side, the body for the diagnosis of disease. The nuclear images help in visualizing, characterizing and quantifying the progress and stages of diseases like cancer. In this manuscript the role of nuclear medicine in the evaluation of acute diseases using nuclear imaging techniques and its disposal has been discussed.

Keywords: Nuclear medicine, radiopharmaceuticals, radioisotopes, nuclear imaging, radiation hazard, radiation disposal

INTRODUCTION

Millions of people are infected by different chronic *diseases* irrespective of sex, age, region or country. These diseases require accurate and timely diagnosis and treatment. With the advent of science and technology several newer medicines along with tools and techniques have been evolved over time providing high-quality healthcare services. The discovery of radioactivity by Henri Becquerel and Marie Curie in 20th century paved the path towards modern day novel health care system. Advancement in the use of radio-isotopes other than atomic energy production comes up as a savior to trace, diagnose and treat

various chronic diseases. Isotopes are distinct nuclear species of the same chemical element that contain same number of protons but different number of neutrons in their nuclei. Isotopes are of stable and unstable type where the unstable isotopes are termed as radio isotopes which changes over time with the emission of alpha or beta particles to transform into more stable form. Moreover, all decaying process of radioisotopes emits gamma radiation. Radio-isotopes are effectively used in health-care, industrial applications, environmental studies etc. The use of radio-isotopes or radionuclides in health-care system is termed nuclear medicine (Cherry,

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Sorenson and Phelps, 2012). There are around hundred radio-isotopes are used as nuclear medicine based on its suitability in different organs of the body (Krane, 1955). Soon after the discovery of X-ray by Roentgen in 1895, medical science made use of this technology in diagnosis [Fig. 1]. Radiology techniques for repairing fractured bones result from X-rays' ability to pass through soft tissues with ease. X-rays are attenuated when they come into contact with bones. The radiopharmaceutical study commonly requires Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) to trace the radioisotopes using emitted gamma radiations to form images for better visualization of internal organs at a molecular level (Kong, Zhu and Yang, 2024). The nuclear imaging gives images of both tissue and bone as well as provides their functions, unlike X-rays which gives only the image of bone. The radioactive doses are given to the patient by injection, inhalation or orally and images are captured using gamma rays' detection cameras, Positron Emission Tomography (PET)/ computerized axial tomography (CAT) scanners, and other specialized equipment to provide information regarding variety of diseases.

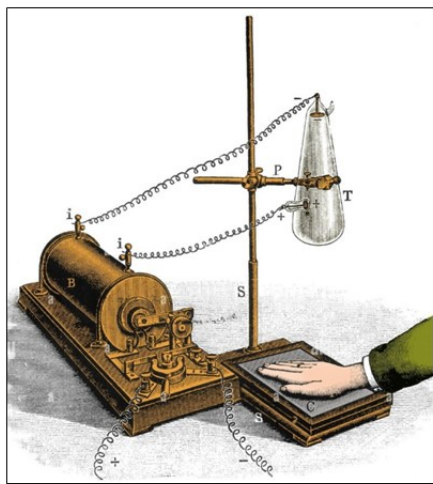


Fig. 1: Representative image, Roentgen X-ray discharge tube (<https://www.alamy.com/stock-photo-wilhelm-conrad-roentgen-1845-1923-german-experimental-physicist-and-104020180.html>)

Tomography is an imaging technique which uses internally, inserted radio-isotopes or externally incident X-rays to image a specific slice of the body's interior anatomy. It has seen some of the most notable advancements in imaging techniques. These days, a lot of X-ray photons are passed across the area of interest to provide tomography images. Since the X-ray beam typically scans in a circle around the patient, a computer is required for these reconstructions, which is known as computerized axial tomography, or CAT. In PET isotopes such as ^{15}O , ^{13}N , ^{11}C , and ^{18}F having half-lives 2 min, 10 min, 20 min, 110 min respectively are used. Since all of these isotopes have brief half-lives, a cyclotron is required for their production, and one must be located near the diagnostic facility. PET has benefits over CAT. While PET scans of the brain can show dynamic effects like blood flow, CAT scans are static.

Nuclear Magnetic Resonance (NMR) imaging is the most recent advancement in the field of imaging. In nuclear magnetic resonance (NMR), a sizable static magnetic field and a time-varying radiofrequency (rf) field perpendicular to the static field are applied to the sample simultaneously. Protium (^1H) an isotope of hydrogen is found abundantly in human body. Resonance with ^1H can be used for medical purposes for imaging. Other stable isotopes like ^{17}O and ^{13}C has an own resonance frequency because of its nuclear magnetic dipole moment. In NMR imaging the patient are not subjected to ionization radiation. Thus, NMR imaging approach offers advantage over gamma, X-ray and PET imaging modalities. Nuclear radiation is mostly used to destroy undesired or malfunctioning tissue within the body, such as an overactive thyroid gland or a malignant tumor. This impact stems from nuclear radiation's capacity to ionize. Radiopharmaceuticals are directed to the patient through inhalation, injection or ingestion (Ramamoorthy, 2018). The radioactive material travel to specific spot in the patient's body and release gamma ray. The emitted gamma rays are then detected and recorded with the aid of gamma cameras. The concentration of radiative material and its distribution within the body is

obtained from the images obtained. Thus, nuclear medicine is used to evaluate blood flow to the heart muscle, bone cracks and to detect thyroid gland for any anomalies. It is used to detect blood clots or other conditions in the lungs, inflammation or infection in the body and to diagnose and monitor conditions like epilepsy, dementia, certain tumors and diseases like cancer. This study provides an overview of nuclear medicine while highlighting its evolution, mechanisms, challenges, and future potential.

Mechanism of nuclear medicine scans

Nuclear medicine scans are irreplaceable for monitoring and diagnosis of diseases like cancer, thyroid disorder, neurological syndrome, heart ailment etc. This scan provides useful information that improves the results of other procedures when comes to patient treatment (Kong, Zhu and Yang, 2023). Nuclear medicine scan not only provides the images of the body but also delivers evidence concerning the functioning of the organs or the tissues involved (Anderson 2019). The information collected through a nuclear medicine scan provides a comprehensive view of the patient's well-being and also complements other imaging techniques used. The principle of its operation is to detect and measure gamma radiations emitted by radioactive elements known as radiopharmaceuticals which are introduced in

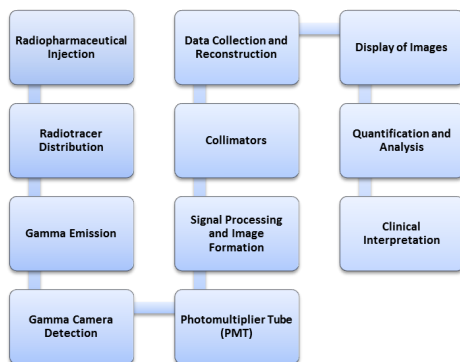


Fig.2: Mechanism of nuclear medicine scans

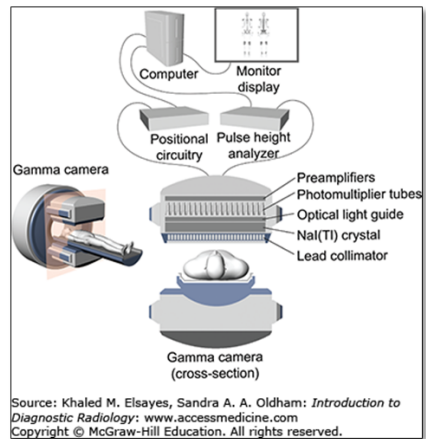


Fig. 3: Components of Gamma Camera
([https://accessmedicine.mhmedical.com/content.aspx?bo/p\]/pop-666666-2](https://accessmedicine.mhmedical.com/content.aspx?bo/p]/pop-666666-2))

the patient's body. The schematic analysis of the mechanism of nuclear medicine scan is shown in Fig. 2 and the main components of Gamma camera which are responsible for image processing and display are depicted in Fig. 3.

The scanning technique's drawback is that it only measures a small percentage of the decaying activity at a time; the remainder is lost and adds to the patient's needless radiation exposure.

Radiation Exposure

Medical imaging (Parker and Patton 2013) is the key cause of radiation exposure. The absorbed dose of ionizing radiation in living tissues are quantified by means of units such as sieverts (Sv) and millisieverts (mSv). The radiation levels due to natural background can differ significantly and it is reliant on the area of living. For instance, some regions have higher radiation levels due to ecological factors while other may have low. The usual background radiation exposure from natural sources is typically about 1 to 3 mSv per year. During Chest X-ray the radiation exposure met by a patient is nearly 0.1 mSv per procedure. However, during CT scan of the abdomen or pelvis the exposure

might range from 10 to 20 mSv depending on the explicit etiquette and part being scanned. For Breast cancer scanning the mammogram necessitates not more than 0.5 mSv per examination. For radiation workers the annual occupational exposure limit is usually set at 20 mSv averaged over five years, with no year surpassing 50 mSv. This is followed in many nations. For the general public, the annual exposure limit is typically set at 1 mSv per year above background levels. In radiation therapy much higher dose is used compared to diagnostic procedures. Doses are carefully calculated and administered to the specific target inside the body which is affected by cancer. The doses used in radiation therapy are much higher than those used in diagnostic procedures. Dose is carefully calculated and directed to the target specific parts of the body which is affected by cancer. The total dose that can be administered in a body can range from a few grays to tens of grays (where 1 Gy = 1000 mSv). While in most diagnostic procedures the dose ranges from 1 to 10 mSv. Nevertheless, there can be exclusions also depending on the definite radiopharmaceutical and the practice. If the radiation level overdoes 1 mSv above background levels, evacuation is typically considered in the instance of nuclear emergency. Due to the absence of earth's shielding atmosphere astronauts traveling to the International Space Station (ISS) and out there are exposed to higher levels of radiation. This can range from a few mSv for a stay on the ISS to possibly several hundred mSv for deep space missions.

Radiation hazards associated with nuclear medicine

There is a potential radiation hazards linked with nuclear medicine although it is cautiously regulated and used in controlled settings. In the procedures discoursed above the radioactive materials used expose patients and healthcare workers to radiation hazards, together with probable long-standing dangers such as cancer and genetic mutations. To minimalize the

risk of radiation hazard and to ensure that the exposure remains inside safe limits, strict protocols and safety regulatory rules has to be maintained. The energy confined in ionizing radiation can be used to disrupt free electrons from atoms that are closely associated. This might cause possible damage to living tissues and henceforth the main concern in nuclear medicine is the potential exposure of patients, healthcare workers, and the environment to ionizing radiation emitted by the radiopharmaceuticals. With time the radioactivity of the radiopharmaceuticals decreases i.e. the half-life of radiopharmaceuticals is particular. Nuclear medicine professionals follow strict protocols to lessen radiation exposure. This comprises using the minimum operative dose of radiopharmaceuticals and taking protections to safeguard and contain the radioactive material. For prenatal or breastfeeding patients' different protection measures are set aside. During pregnancy unless absolutely essential nuclear medicine treatments are avoided but if done, breastfeeding is discontinued for a while. The risks connected with the prudent use of radioactive materials are generally balanced by the benefits of nuclear medicine in recognizing and treating certain medical conditions. Cautious adherence to legal necessities and safety measures is crucial to ensure that radiation risks are kept to the minutest.

Disposal of Nuclear Medicine

Disposing of nuclear medicine waste is another serious aspect, demanding adherence to strict rules to avoid environmental contamination. The disposal of radiopharmaceuticals is carefully regulated to ensure the safety of both humans and the environment (IAEA, 2005). The general method of disposal includes decay-in-storage for short-lived isotopes, sewer removal of patient excretions, and transmission to certified services for longer-lived radioactive elements. Proper disposal helps to mitigate the risks associated with the use of radioactive substances in medical sceneries. The general steps and considerations for

the disposal of nuclear medicine (Fig. 4) are discussed below:

A) Identification and Segregation: Identification and Segregation is the primary phase in the disposal of nuclear medicine. Nuclear medicine constituents should be stored in a selected zone away from non-radioactive materials. The zone of storing must be marked cautiously and entry to it should be provided only to the authorized employees. Labelling with specific identification and information is to be made in all packages, containers and vials containing the radioactive material. Details including the activity, date of preparation, and any required radiation warning symbols are to be provided on the labels. To visually differentiate containers of radioactive materials from non-radioactive one's stickers or color-coded labels may be used. This may help in preventing accidental mixing of materials. To store radioactive materials containers made of suitable shielding materials such as lead which is non-reactive can be used. The lead containers are designed to prevent leakage and act as shield against emitted radiation. Detailed record and accuracy must be maintained for each batch of radiopharmaceuticals including relevant quality control tests. Geiger-Muller counters, scintillation detectors, or other radiation monitoring devices are used to verify the presence and level of radiation around containers and storage areas. Periodic inspections and audits of the storage area and containers help ensure that segregation and identification practices are being followed correctly.

B) Radiopharmaceutical Decay: Radioactive nuclei are unstable. They stabilize themselves by emitting α particles, β particles and γ radiations (Knoll, 2010, Martin, 2019, Serway and Jewett 2018). In nuclear medicine, radiopharmaceutical decay is a natural process in which the radioactive elements decay over a particular period of time. The decay occurs due to the emission of ionization radiation from the unstable nuclei present in the

radiopharmaceutical. The half-life ($T_{1/2}$) of the radioactive element is the time required for half of the element to decay. It is a characteristic property of any radionuclide and may vary from fraction of a second to millions of years. With the decay of radionuclide, the total number of atoms present in the sample decrease resulting in the reduction of activity. The power of the released radiation also declines over time. So, in therapeutic nuclear medicine based on the wanted extent of treatment, the radio nuclides with precise half-lives are selected. Technetium-99m has a short half-life of approximately 06 hours and Iodine-131 has a comparatively long half-life of around 8 days which is why it is used in the treatment of thyroid cancer. Once a radiopharmaceutical has decayed to a harmless level, the resulting waste is managed according to conventional radioactive waste disposal measure. This guarantees that there is no damage to the environment or public health from the waste. The use and handling of radiopharmaceuticals in nuclear medicine are subject to strict regulatory oversight by organizations such as the Nuclear Regulatory Commission (NRC) in the United States (NRC, 2020). This ensures that safety protocols are followed for safe disposal.

C) Authorized Radioactive Waste Disposal Facilities: There are authorized facilities well equipped to contain and handle radioactive materials safely (Ravichandran, Binukumar, Sreeram, and Arunkumar 2011). Disposal of radioactive waste, including outdated or unused radiopharmaceuticals, must be done appropriately through those authorized facilities. During transportation of radioactive waste to disposal facilities extreme care must be taken and certified transporters must be involved who are competent to handle radioactive materials. The disposal should be in accordance with local and national regulations. Radiopharmaceuticals should be securely packaged in containers that are designed to prevent leakage or breakage during transport. Detailed records of the type, quantity, and

disposal of radioactive waste should be maintained for regulatory compliance and auditing purposes. Before and after disposal, the disposal area should be monitored for any residual radiation to ensure that no contamination has occurred. As we have discussed earlier that there are specialized facilities equipped to handle radioactive materials, certain types of solid radioactive waste which are contaminated with low-level radioactive substances, may be burned in that case. Liquid radioactive waste may further undergo treatment processes to reduce its radioactivity or to separate the radioactive component for further processing or disposal. The Atomic Energy Regulatory Board (AERB) sets severe adherence to safety etiquettes and strategies during trials and disposal and directs the nuclear medicine procedures in India to tune with it. The main authority to monitor and supervise the usage of ionizing radiation, included in nuclear medicine trials is the AERB, under the Department of Atomic Energy (DAE). These trials are in position to confirm that both patients and healthcare specialists are exposed to the lowest possible levels of radiation while still gaining precise analytical data or therapeutic benefits. Nuclear medicine follows the principle of ALARA

(As Low As Reasonably Achievable). This means that every single effort is made to curtail radiation exposure while still gaining the obligatory diagnostic or therapeutic statistics.

Discussion and Conclusions

Scientific improvement and innovative paths of enduring research has vastly contributed to the field of nuclear medicine which is evolving incessantly. To further improve diagnostic precision and treatment efficacy new radiopharmaceuticals and imaging techniques are being developed. Nuclear medicine delivers thorough diagnostic evidence which is extremely accurate and which might not be obtained through other imaging modes. Nuclear medicine is vital for treating an extensive range of ailments, even outside diagnosis, including certain forms of cancer and hyperthyroidism. It is used to selectively mark and terminate abnormal cells. In conclusion, nuclear medicine is a vital constituent of contemporary healthcare, contribution a variety of diagnostic and therapeutic competences. Its importance in present-day medical practice is highlighted owing to its capability to deliver accurate information at the molecular level,

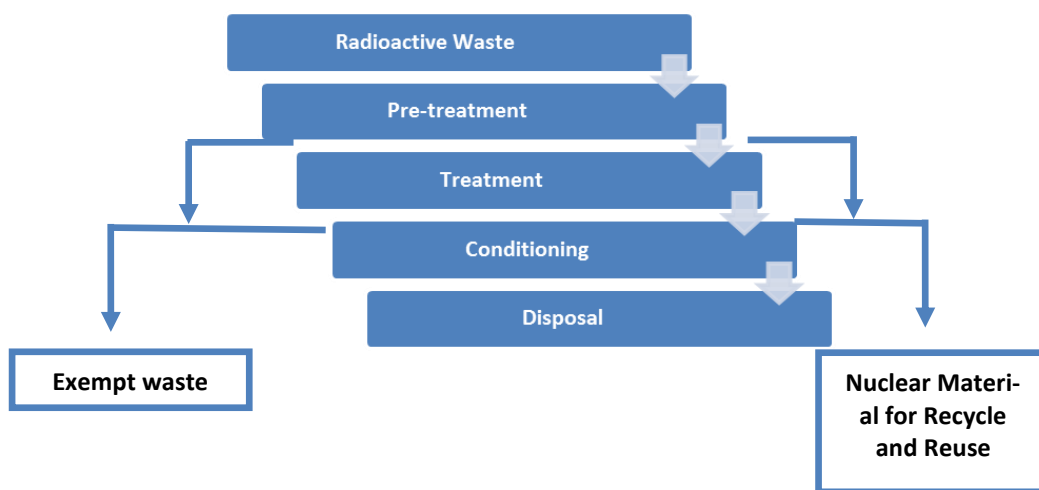


Fig. 4: Schematic steps involved in the disposal of radioactive waste

coupled with improvements in treatment opportunities. Even though nuclear medicine has countless benefits, it also encounters challenges. It can be expensive, non-accessible and there are regulatory issues concerning the management and disposal of radioactive elements.

There is a need for a holistic approach from government agencies and professionals in implementing and following the standard norms in the utilization and scientific disposal of nuclear waste. The enduring technological improvements embrace promise for the future and potential leading to additional cheap, harmless, and accessible treatments that improve patient care even better.

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Conflict of Interest

The authors declare no conflicts of interest.

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A Study on The Integrated Farming Approaches in Bharaliporiya Kanyaka Bahumukhi Pam and Pabhoi Greens

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ABSTRACT

This study explores the integration of pig-fish, duck-fish and paddy-fish farming systems with a focus on their economic and ecological sustainability. Conducted at two farms in Assam, India, the research evaluates the role of pig and duck manure in enhancing pond productivity through nutrient cycling. Data were collected through surveys, questionnaires, and personal interviews with farm staff and management. Key physico-chemical parameters such as pH, dissolved oxygen, alkalinity and turbidity were monitored to assess water quality. This study examines pre-stocking pond management for integrated farming systems (rice-fish, pig-fish, duck-fish). Pond preparation involved dewatering, fertilization with organic and inorganic fertilizers, and species selection based on compatibility, growth, and environmental tolerance. Duck and pig manure was used as organic fertilizer, with different application methods to enrich pond water. The rice-fish system enhanced soil fertility and controlled pests. Water quality parameters were monitored, indicating favorable conditions for fish. Fish species like *Catla catla* and *Cyprinus carpio* were used, promoting sustainable, productive integrated farming. The study concludes that integrated farming is a cost-effective, sustainable approach for rural agricultural development, with potential to enhance food security and farmer income when properly managed.

Keywords: Aquaculture, Integrated fish farming, Bharaliporiya Kanyaka Bahumukhi Pam, Pabhoi greens

INTRODUCTION

Aquaculture ranks among the world's fastest-growing food production sectors, with its commodities expected to see rising demand and greater significance in the future (Pedini & Shehadeh, 1997; FAO, 2002; Delgado et al., 2003). Asia has been the hub of global aquaculture production for decades, contributing over 90% of the total output, with China leading as the largest producer (Gangadhara et al., 2016). In India, water bodies such as ponds, lakes, and tanks suitable for

fish farming cover approximately 2.34% of the country's total land area (Kumar et al., 2022).

Integrated fish farming involves the combined management of aquaculture, agriculture, and livestock, where by-products from one sector become inputs for another (Kalita, 2017). For instance, fish waste can fertilize crops, while crop residues can be used as fish feed (Chandra et al., 2023). Animal manure added to fish ponds supports the growth of photosynthetic organisms, enhancing productivity (Saikia et al., 2020).

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Although commonly referred to as “waste,” these by-products are better considered “resources out of place” (Taiganides, 1978). However, careful management is essential to prevent the introduction of antimicrobial-resistant bacteria through manure and excess feeding (Saikia et al., 2020). Nutrients like nitrogen (N) and phosphorus (P) from livestock manure act as natural fertilizers, promoting aquatic food webs. While these elements enhance fish pond productivity, some farms also use slaughterhouse waste as fish feed for carnivorous species (Little & Edwards, 2003). Integrated fish farming offers economic and environmental advantages, being cost-effective, climate-resilient, and requiring minimal initial capital (Chandra et al., 2023).

The integrated fish farming systems combining rice, ducks, and pigs have proven to be effective and sustainable agricultural practices, particularly in regions of Southeast Asia and parts of India. These systems offer multiple benefits, such as enhanced fish production and improved soil fertility, by using animal manure as fertilizer. For example, ducks contribute by controlling pests like aquatic weeds and mosquito larvae, while their droppings serve as an important organic fertilizer for fish ponds, promoting better growth of fish and aquatic plants (Gebu, 2021).

In Assam, integrated fish farming has gained widespread popularity. Bharaliporiya Kanyaka Pam emerged as a transformative initiative addressing regional brain drain while fostering local self-reliance. This visionary project, spearheaded by Shri Padma Hazarika, reflects a deep commitment to community development and sustainable agriculture (kanyakapam.com).

Similarly, Pabhoi Greens, also known as Lakshmi Agriculture Multipurpose Project, operates as a certified organic farm in Biswanath, Assam. Founded by Mr. Neelam Dutta, it boasts an extensive integrated fish farming system covering half its farmland. The farm breeds carp, catfish, and anabas while ensuring genetic purity through selective and single-breeding practices. With eco-hatcheries and organic fish feed production, Pabhoi Greens produces 8,000 kg of

mature fish, 8.5 million spawn, and 600,000 fish fry and fingerlings annually (pabhoigreens.com).

Despite their success, systematic studies on these farms' operations remain limited. This research aimed to explore integrated fish farming practices at Bharaliporiya Kanyaka Bahumukhi Pam and Pabhoi Greens, focusing on the role of pig manure, duck droppings, and paddy cultivation in enhancing fish growth and pond productivity.

MATERIALS AND METHODS

Study Area

The study was conducted from March to June, 2024 at two sites in the Sonitpur district of Assam, selected for their distinct agro-climatic conditions: Jamugurihat and Biswanath.

Site 1: Bharaliporiya Kanyaka Bahumukhi Pam

Located at latitude 26.6770°N and longitude 92.6984°E, about 8 km from Jamugurihat's main town, Bharaliporiya Kanyaka Bahumukhi Pam spans 800 bighas of water bodies dedicated to integrated fish farming involving ducks and pigs. The farm also operates a large dairy producing curd, milk, cream, ghee, butter, and sweets. A mini dairy plant established in 2021 improved transparency and fairness in operations.

Extensive rice and vegetable cultivation further supports fish farming, with rice waste repurposed as fish feed. The farm includes 26 large ponds, two hatching pools, and 26 nursery tanks. Under the leadership of MLA Shri Padma Hazarika, the farm has grown into a community-driven enterprise supported by stakeholders, consumers, and well-wishers. In addition, a poultry project launched to produce 20,000 eggs daily aims to create jobs and position Jamugurihat as a leading egg producer in Assam.

Site 2: Pabhoi Greens

Situated at latitude 26.8518°N and longitude 93.3389°E, approximately 18 km from Biswanath's main town, Pabhoi Greens operates as a certified organic farm promoting agro-tourism and farm stays. The farm produces organic seeds and provides free training on organic seed production to local farmers.

Pabhoi Greens maintains a gene pool of

108 indigenous rice varieties from India and Southeast Asia, producing commercial rice types such as Joha, black rice, sticky gum rice, and soft rice. Half of the farm's land supports fish breeding, featuring species like carp, catfish, and anabas. The farm has eight nursery tanks, ten rearing tanks, nine stocking tanks, six brood tanks, and eco-hatcheries.

Integrated fish farming methods include rice-fish and duck-fish systems. The farm's 38 cows and 50 ducks play essential roles in organic milk production, vermicomposting, and bio-pesticide creation, with duck waste also contributing to the fish farming system.

Survey Method

To conduct the study, formal permission was secured from the owners of both farms. Data collection was carried out using a combination of standard questionnaires and in-depth personal interviews with farm staff and management. This dual approach ensured a comprehensive understanding of farm operations, management practices, and integrated farming techniques.

Structured questionnaires helped gather quantifiable data on farm productivity, pond management, and species selection, while personal interviews provided qualitative insights into farming challenges, manure management strategies, and ecological sustainability.



Fig. 1: Detection of male and female in fishes

RESULTS

Pre-stocking Pond Management

At both farms, fishery ponds were prepared by reinforcing and trimming the dykes. Planning for integrated farming systems—rice-fish, pig-fish, and duck-fish—was based on pond size. To enhance pond fertility and remove unwanted fish species, ponds were dewatered before stocking.

Fertilization involved both organic and inorganic methods. Organic fertilizer, specifically cow dung, was applied one week after dewatering. Inorganic fertilizers, including urea, super phosphate, and potash, were added 7-15 days after the organic fertilizer application. Fish were introduced into the pond water 7-15 days after the inorganic fertilizer application.

Selection of Fish Species

Specific criteria guided the selection of fish species:

- **Compatibility:** Selected species needed to coexist well with one another.
- **Growth Rate:** Fast-growing species were prioritized.
- **Feed and Stocking Ratio:** Species combinations were adjusted according to available feed resources.
- **Disease Resistance:** Fish species resistant to common diseases and parasites were preferred.



Fig. 2: Injection of breeding hormone

- **Environmental Tolerance:** Species that could withstand high organic content and low oxygen levels in the water were chosen.

The male and female fishes are detected first based on their pectoral fin colour and texture as well as by belly pressing. Then an injection containing the gonadotropins are injected to promote breeding. Fig. 1 and 2 shows the detection of fish gender and hormone injection in Pabhoi Greens.

Construction of Duck Rearing Facilities

Most duck houses are constructed on pond dykes rather than directly over the water to reduce construction costs and simplify management. These shelters provide shade from the sun and protection from heavy rain, while allowing proper air circulation. Side curtains are essential to shield the ducks from wind-driven rain.

Locally available materials such as bamboo for the frame, bamboo matting for the floor, and straw for the roof are commonly used in duck house construction. Ducks can be housed and

fed either on floating rafts or movable structures built over the pond. In both setups, duck faeces and uneaten food fall directly into the pond, enriching the water with nutrients.

Duck houses must be well-protected against predators, with a height of 4-5 feet. The required space depends on the number of ducks, with each bird typically needing about 4 square feet of space.

When ducks are housed on the dyke, only 50-60% of their manure enters the pond water. However, housing ducks directly over the pond eliminates manure collection, storage, and transportation costs, reducing the risk of environmental pollution. Despite these benefits, duck movement can cause significant erosion of the pond's edges, leading to dyke destruction and increased water turbidity. This siltation reduces light penetration, hindering photosynthesis and affecting the pond's ecological balance. Fig. 3 shows the ducks swimming in the integrated duck cum fish culture ponds and Fig. 4 shows a typical duckery.



Fig. 3: Ducks swimming in the pond.



Fig. 4: View of a duckery

Methods of Pig Manure Application

At Kanyaka Bahumukhi Paam, pig manure is applied using two methods: direct and indirect application. In the direct application method, the pigsty is built on the pond embankment, allowing pig excreta to be washed directly into the fish pond. This method is commonly used in small-scale farms. Indirect application allows the pigsty to be constructed at a convenient location on the farm, and pig dung is collected in a large storage tank. The manure is then applied to the fish pond in controlled quantities and at scheduled intervals. This method is typically employed in large-scale farms.

No pigsty facilities were found at Pabhoi Greens.

Construction of Pigsty

The pigsty floor consists of two layers. The lower layer is made of sand, gravel, and cement for

stability. The upper layer is constructed with brick soiling and rough cement mortar for durability.

A brick-and-cement delivery pipe is attached to the pigsty, channelling waste and wash water directly into the fish pond. Feeding and drinking troughs are alternately positioned along the sidewalls of each compartment for easy access. The pigsty's sidewalls are constructed using high-quality 75.0 mm thick bricks, with iron rods reinforcing the upper sections. Each compartment has a gate allowing pigs to move in and out as needed. The roof, made of tin sheets, shields the pigs from direct sunlight. Pigs remain inside the pigsty and are fed a balanced diet to ensure proper nutrition and productivity. Fig. 5 and 6 show the inner and lateral views of a pigsty. Fig. 7 shows excreta and waste pipe from the pigsty to the fish pond.



Fig. 5: Inner view of pigsty



Fig. 6: Lateral view of pigsty construction



Fig. 7: Excreta and waste pipe from the pigsty to the fish pond

Integrated Management of Rice and Fish Farming

In the rice-fish farming system, various fish species are introduced into paddy fields, creating a mutually beneficial environment. Rice cultivation enhances soil fertility by increasing nitrogen and phosphorus content, improving water aeration, and aiding in pest control. Simultaneously, fish benefit from the nutrient-rich water and feed on plant roots, algae, and aquatic weeds.

The fish also help control mosquito larvae, algae growth, and aquatic weeds, maintaining ecological balance. In return, rice plants provide shade, reduce water temperature, and create favourable conditions for fish during summer.

To sustain fish farming during the rice harvest and dry seasons, channels are constructed within the flat paddy fields, ensuring continuous fish growth. Commonly cultivated fish species in this system include *Catla catla*, *Clarias batrachus*, and *Channa striatus*.

Preparation of Fish Feed

Fish are fed using locally available organic ingredients such as rice bran, mustard oil cake, fish meal, and molasses. These components are mixed in a specific ratio of 2:2:0.5:0.25, ensuring a balanced nutritional profile.

The prepared mixture is shaped into balls and directly thrown into the pond as feed. Another method involves cooking the mixture, packing it into small bags, and puncturing the



Fig. 8: Preparation of fish feed.



Fig. 9: Making of holes in the food packet



Fig. 10: Binding of the food packet with the bamboo stick in the centre of the pond

bags with holes to allow fish to access the feed. Fig. 8 and 9 show the preparation of fish feed and making of holes in the food packet. The packets are then attached to bamboo sticks placed in the centre of the pond for easy feeding, which is shown in Fig. 10.

Estimation of Excreta Produced by Ducks and Pigs in Cage Conditions

Duck droppings and pig excreta are valuable organic fertilizers commonly used in integrated fish farming systems. Monthly analyses of waste production revealed that a single duck produces between 110g and 200g of droppings per day. Similarly, a pig generates an average of 1 to 1.5 kg of excreta daily. To enhance pond productivity, a mixture of duck droppings and pig manure is prepared and applied as organic fertilizer.

Proximate Composition of Manure

The chemical composition of duck and pig manure was analysed at the farms. The duck manure was found to contain total Nitrogen 0.87%, Phosphorus 0.46%, Potassium: 0.32% and moisture Content 49.7%, while the pig manure contains total Nitrogen 1.12%, Phosphorus: 0.61%, Potassium: 0.38% and moisture content 68.2% (Fig. 11). These

nutrient-rich manures contribute to maintaining pond fertility, supporting plant and aquatic life essential for fish farming productivity.

Physico-Chemical Characteristics of Pond Water

The physico-chemical characteristics of pond water are crucial for the growth and reproduction of fish. These characteristics help maintain the equilibrium of the aquatic environment, and fish serve as indicators of environmental disturbances. Proper management of water quality is essential for the healthy development of aquatic flora and fauna. Key water parameters of both the farms are presented in Fig. 12.

3.10.1. Hydrogen Ion Concentration

(pH)

pH is a critical factor that affects the growth and reproduction of aquatic organisms. It measures the acidity or alkalinity of the water on a scale of 0 to 14, with 7 being neutral. pH is closely linked to the concentration of carbon dioxide in the water. As the timing of water sample collection can influence the pH result, accurate measurements are essential. In this study, the pH was found to be 6.5 at site 1 and

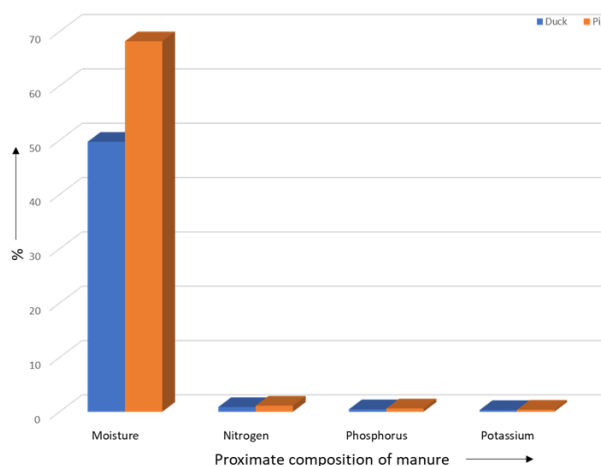


Fig. 11: Proximate composition (%) of Duck and Pig manure analysed in both Kanyaka Bahumukhi Pam and Pabhoi Greens

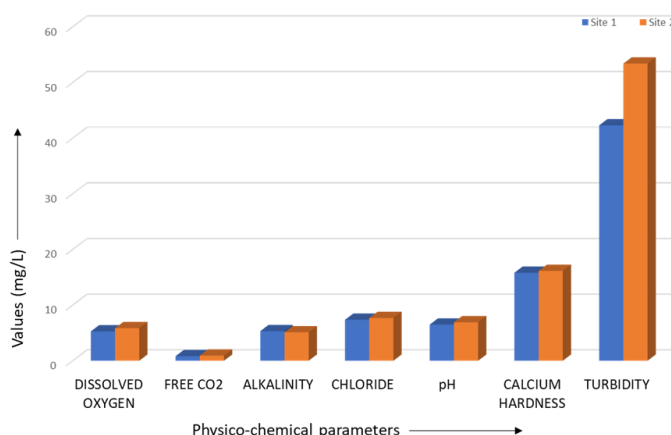


Fig. 12: Physioco-chemical parameters of pond water of both Kanyaka Bahumukhi Pam and Pabhoi Greens. Values are expressed in mg/L.

6.91 at site 2 on January 12, 2024.

3.10.2. Dissolved Oxygen (DO)

Dissolved oxygen is vital for the aerobic metabolism of aquatic organisms, playing a key role in water quality. Adequate oxygen levels are essential for the growth of fish and other organisms. At site 1, the DO was 5.28 mg/L, and at site 2, it was slightly higher at 5.86 mg/L.

3.10.3. Total Alkalinity (TA)

Alkalinity refers to the presence of minerals that neutralize acids in water, impacting the overall health of the aquatic ecosystem. Total alkalinity is measured in parts per million (ppm) or

milligrams per liter (mg/L) of calcium carbonate (CaCO_3). A healthy range for total alkalinity is between 4-200 mg/L. At site 1, the alkalinity was measured at 5.32 mg/L, while at site 2, it was 5.11 mg/L, showing similar levels.

3.10.4. Turbidity

Turbidity refers to the cloudiness of water caused by particles suspended in it. Increased turbidity reduces light penetration, which can negatively affect aquatic organisms. The turbidity level at site 2 was higher at 53.4, compared to 42.3 at site 1, indicating greater cloudiness at site 2.

Table 1: Percentage of introduction of different fish species in the three modes of integrated fish farming in both Kanyaka Bahumukhi Pam and Pabhoi Greens

Name of Fish Species	% of Introduction		
	Paddy cum Fish	Duck cum Fish	Pig cum Fish
<i>Cyprinus carpio</i>	50	15	20
<i>Labeo gonius</i> + Catfish	10	5	15
<i>Labeo rohita</i>	20	15	10
<i>Cirrhinus mrigala</i>	10	10	10
<i>Puntius sp.</i>	10	5	0
<i>Catla catla</i>	0	20	20
<i>Hypophthalmichthys molitrix</i>	0	15	10
<i>Ctenopharyngodon idella</i>	0	10	10

Fish Species in Integrated Fish Farming

Various fish species are used in the integrated systems of rice-fish, duck-fish, and pig-fish farming. The fish species involved include *Cyprinus carpio*, *Labeo gonius*, catfish species, *Labeo rohita*, *Cirrhinus mrigala*, *Puntius* sp., *Catla catla*, *Hypophthalmichthys molitrix*, and *Ctenopharyngodon idella*, each contributing to the system in different proportions. The specific names and percentages of these fish species in the various integrated farming systems are illustrated in the Table 1.

DISCUSSION

The construction of duckeries and pigsties plays a crucial role in integrated farming systems, particularly in aquaculture, where animal waste is used as an organic fertilizer for fish ponds. In the case of duck farming, duck houses are typically built on pond dykes to minimize construction costs while maximizing efficiency. These structures provide shade and protection from the rain, and the ducks' waste is directly deposited into the pond, enriching the water with essential nutrients for fish growth. According to studies, duck houses can be constructed using locally available materials like bamboo and straw, and their height typically ranges from 4 to 5 feet, providing sufficient space for each bird to thrive. However, research also suggests that excessive duck density in ponds can lead to over-fertilization, potentially harming fish stocks (Gebru, 2021).

For pig farming, pigsties are built in proximity to the pond to facilitate the direct application of pig manure into the water. The structure of the pigsty usually includes a two-layered floor made of sand, gravel, and cement, with a delivery pipe that channels the manure directly into the pond. Pig waste acts as a natural fertilizer, providing nutrients like nitrogen and phosphorus to support pond productivity (Gebru, 2021) (Sathoria and Roy, 2022). However, studies have noted that, while the use of pig manure boosts fish production, it is essential to carefully manage the quantity to avoid water contamination and

oxygen depletion (Sathoria and Roy, 2022).

In the Indian context, integrated systems such as rice-fish-pig and rice-fish-duck farming are increasingly being adopted in various states, including West Bengal, Bihar, and Assam. These practices not only help in managing organic waste but also increase the overall productivity of small farms. In the northeastern states of India, particularly in Assam, integrated farming systems are gaining popularity for their sustainability and low cost. However, the challenge lies in ensuring proper management of manure application, as excessive waste can disrupt the ecological balance of the pond and harm aquatic life (Sathoria and Roy, 2022). Our findings corroborate these observations, suggesting that while these systems offer significant benefits, careful monitoring of animal waste and pond conditions is critical to their long-term success.

The role of duck and pig excreta in integrated fish farming is crucial, as these manures serve as organic feed for many fish species. Estimating the rate of excretion from ducks and pigs is essential to maintaining a balanced fish farming system. Sharma et al. (1979) reported that one pig excretes approximately 1.6 kg of waste per day, while Jhingram and Sharma (1980) estimated duck droppings to be around 145g per day. On the other hand, Bora et al. (1990) estimated pig excreta at 0.82 kg per day. In the current study, pig excreta were found to average between 1 to 1.5 kg per day, while duck droppings ranged from 110g to 200g per day. These results are comparable to the studies referenced, with a slight increase in the amount of duck droppings noted.

The physico-chemical parameters of the pond water were observed to fluctuate considerably across different locations. Key parameters such as pH, dissolved oxygen, alkalinity, and turbidity are essential for maintaining a healthy aquatic environment. The importance of pH in determining pond productivity has been widely acknowledged (Hickling, 1971; Jhingram, 1991). Studies by Das

(1961) and Banerjee (1967) suggested that slightly alkaline water is ideal for fish production, although maintaining the desired pH levels during the monsoon season is challenging. In this study, pH levels were found to be 6.5 at site 1 and 6.91 at site 2 on January 12, 2024.

Dissolved oxygen (DO) is another crucial factor for the aquatic ecosystem, supporting the aerobic metabolism of organisms. It plays a major role in determining water quality. Similar to pH, DO levels showed variations across sites. Sharma and Das (1988) found DO levels fluctuating between 2.6 mg/L and 8.6 mg/L. In the present study, DO levels were 5.28 mg/L at site 1 and 5.86 mg/L at site 2, which is within the mentioned range.

Total alkalinity (TA) is closely linked to pH levels, as water with low pH tends to have low alkalinity. Alkalinity is important for the productivity of aquatic organisms. According to Philipose (1959), water productivity depends on its alkalinity, categorized into low, moderate, and high levels. The study found the alkalinity of the pond water at site 1 to be 5.32 mg/L and at site 2 to be 5.11 mg/L, indicating similar alkalinity levels at both sites.

Turbidity is another important physico-chemical parameter that affects fish growth. Organic and inorganic materials such as clay and suspended particles contribute to turbidity, which can impede sunlight penetration and affect aquatic life. Jhingram (1991) emphasized that excessive turbidity, particularly from silt or mud, can harm fish, while turbidity caused by plankton growth indicates a fertile pond. In the present study, turbidity was lower in the pig-treated pond at site 1 (42.3), compared to the duck-treated pond at site 2 (53.4).

In terms of fish species, various species like *Cyprinus carpio* (common carp), *Labeo rohita* (rohu), and *Catla catla* (catla) are commonly cultured in integrated rice-fish systems in India. The combination of rice cultivation and fish farming ensures mutual benefits, with fish helping to manage algae and pest control, while rice

provides food for the fish during their growth stages (Sathoria and Roy, 2022). Integrated pig-fish and duck-fish farming systems are increasingly recognized for their sustainability and productivity benefits. These systems involve the strategic use of animal manure—whether from pigs or ducks—as a natural fertilizer for fish ponds. In both pig-fish and duck-fish integration, the manure significantly boosts the growth of plankton and algae, which provide essential nutrients for fish. Studies indicate that the fertilization from pig excreta results in better growth of various fish species such as *Catla catla*, *Rohu*, *Mrigala*, and *Grass carp*, compared to systems without animal manure (Sarm et al., 2022; Cruz and Shehadeh, 1980). However, while pig manure has been shown to yield higher fish productivity than duck manure (Sarm et al., 2022), both systems help in recycling nutrients and reducing the need for synthetic fertilizers, making them both cost-effective and environmentally friendly.

In India, particularly in the states like Assam and West Bengal, integrated systems like these are proving beneficial in rural farming, providing farmers with increased yields and additional income. For instance, in Purulia, West Bengal, integrated duck-fish farming has not only improved fish productivity but also raised the economic status of farmers by reducing their dependence on external feed and fertilizers (Majhi, 2018). While pig-fish systems generally show higher productivity, careful management of manure is essential to avoid issues like over-fertilization, which can harm water quality and fish health (Cruz and Shehadeh, 1980). Our findings align with these observations, suggesting that integrated systems can thrive in smallholder farming, but success depends on proper management of animal waste and ensuring that manure levels do not overwhelm the system.

Some of the previous works on pig farming in integrated systems has often been found to be less profitable. Sharma et al. (1979) reported that the return on variable costs for pig-fish

farming was 68%, with limited profits from pig raising. Similarly, Jhingram and Sharma (1980) found the profitability of pig farming to be low. In contrast, the integrated fish farming systems in the current study showed promising results, providing substantial profits and contributing positively to the entire aquatic ecosystem.

These integrated systems have not only proven to be effective in increasing production yields but also provide significant economic and environmental benefits by reducing the need for external fertilizers and supplements. Overall, these farming models contribute to sustainable food production while improving the livelihoods of farmers, especially in rural and tribal areas where resources are often limited (Gebbru, 2021).

CONCLUSION

In conclusion, integrated fish farming systems involving rice, pigs, and ducks offer a sustainable and productive approach to aquaculture, benefiting both the aquatic ecosystem and local communities. The use of organic fertilizers, such as duck and pig manure, plays a vital role in enhancing pond fertility and supporting fish growth. This study highlights the importance of estimating manure excretion rates, as well as monitoring key physico-chemical parameters like pH, dissolved oxygen, alkalinity, and turbidity, to maintain optimal conditions for aquaculture. The findings from both study sites indicate that proper management of these parameters is crucial for maximizing productivity and ensuring a healthy environment for fish. While pig farming has shown limited profitability in some previous cases, integrated fish farming systems at both sites demonstrated substantial benefits, contributing to the economic upliftment of rural communities. Ultimately, integrated farming technologies are a promising means of improving agricultural sustainability, enhancing food security, and promoting economic growth in rural regions.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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Indian krait venom and its therapeutic potential- A mini review

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ABSTRACT

Venoms, the secretions of venomous animals, are conventionally thought to be the source of toxic substances though the views about venoms in the recent era have been changed. Snake venom is a cocktail of multifunctional biomolecules that has evolved with the purpose of capturing prey as well as for defense. Due to their inherent high affinity and selectivity, many venom components are used as versatile biochemical tools or considered as prototypes of medicines. Bioactive components present in different snake venoms are mainly proteins and peptides either enzymatic or non-enzymatic which have tremendous therapeutic potential and are being used for the treatment of variety of diseases.

Kraits (*Bungarus* sp.) belong to Elapidae family and are widely distributed in East and South-East Asia. Krait venom possesses neurotoxins, membrane toxins, cardiotoxins, three finger toxins, metalloproteinases, cholinesterases, L-amino acid oxidases and serine proteases. The therapeutic potential of krait venom in pathophysiological conditions such as microbial/bacterial and parasitic infections, cancer, arthritis, inflammation, neuromuscular and blood coagulation disorder is discussed in this review. Further thorough research works are required to ascertain the therapeutic potential of krait venom in different diseases.

Key words: *Bungarus* sp., Therapeutic potential, Krait, Krait venom.

INTRODUCTION

Kraits are a group of highly venomous snakes found in parts of tropical Asia from Iran through the Indian subcontinent into the jungles of Borneo. There are 16 recognised species, with eight species from India and several more listed as subspecies. Kraits are nocturnal and typically have a non-aggressive behaviour. Yet, they are among the most medically important snakes, particularly in India, as they have the most toxic venom among terrestrial snakes found on the Indian subcontinent. Cobra and krait species belong to the family

Elapidae and are the most prevalent venomous snakes in India (Ghosh et al., 2016).

Venoms are a cocktail of bioactive molecules, and usually contain proteins and peptides as their main component (Ferraz et al., 2019; Suranse et al., 2018). They are used as a biological/chemical weapon in animals in addition to physical force for predatory purposes and sometimes as a defense mechanism when they are in danger. Animals that have venom include snakes, scorpions, spiders, cone snails and others. Despite the association of snake venom with pain

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and death, translational research has made good use of these venoms for basic science as well as the development of therapeutics inspired by snake venom toxins (Modahl et al., 2020; Koh and Kini, 2012).

Most venomous snakes are found in the Colubridae, Elapidae, Viperidae and Crotalidae families. The major snake bite mortalities are caused by four highly venomous snake species, *Naja naja*, *Daboia russelii*, *Echis carinatus* and *Bungarus caeruleus* or Indian krait which are commonly called as “Indian big four”. These four species of snakes, together, are responsible for over 50,000 reported annual deaths from snakebites in India. Kraits are one of the important members of Elapid family found in Indian subcontinent, North Asia, Southeast Asia, Sri Lanka, Myanmar. In eastern India, two types of kraits *Bungarus fasciatus* (the banded krait) and *Bungarus caeruleus* (the common krait) are common. The common name, krait, stems from the Hindi word “karait”, meaning black. The genus name, Bungarus, stems from the Telugu word, “Bungarum”, meaning gold. One would wonder how the two names for the same group can mean two distinct colours; the answer is that the names go back to the regions where the species were described. It turns out that the banded krait (*Bungarus fasciatus*) with its prominent gold bands was used to coin the genus name, while the word krait must have come from the Hindi heartland of central India where the predominantly black common Indian krait (*Bungarus caeruleus*) is encountered.

Envenoming due to krait (Genus: *Bungarus*) bites is a common, serious health issue in South and South-East Asia. Common krait (*Bungarus caeruleus*) is distributed throughout South Asia, and is responsible for large numbers of cases of severe neurotoxic envenoming each year (Valenta, 2010). It results in a descending flaccid paralysis progressing to life threatening respiratory paralysis unless mechanical ventilation is available (Alirol et al., Pe et al., 1997, 2010, Kularatne, 2002). Krait bites typically occur at night and are

not painful, so many patients do not notice the bite and continue sleeping (Kularatne, 2002; Bawaskar and Bawaskar, 2004; Warrell, 1983), which delays medical care.

“Numerous reports exist of the snake biting sleeping humans in the dead of night. The fact that the bite from this snake is not painful, unlike that of the spectacled cobra or Russell’s viper, adds to the mortality rate as people may not rush to get medical attention,” says Rom Whitaker, renowned herpetologist and co-founder of the Madras Crocodile Bank Trust. Krait venom is neurotoxic and often causes paralysis of muscles, leading to respiratory failure.

Globally snake envenoming is a major cause of morbidity and mortality in the rural tropics (Kasturiratne et al., 2008). Neurotoxicity causing paralysis is one of the major clinical syndromes of snake envenoming, and occurs mainly with Elapids. Despite the magnitude of the health impact of neurotoxic snake envenoming, it continues to be associated with several unresolved issues of clinical importance (Ranawaka et al., 2013). Several reports regarding the therapeutic potential of venom of different elapid snakes and their purified toxins have been found (Mukherjee et al., 2011).

Therapeutic potential of Krait venom:

Therapeutically krait venom had been reported for treatment in different diseased condition. Bungarotoxin isolated from *B. multicinctus*, a member of krait family found in Thailand, Myanmar has been reported to be used in homeopathic treatment of cholinergic disease such as Alzheimer’s due to its ability to inhibit choline uptake (<http://www.scribd.com/doc/29470339/VENOMS>)

Krait venom is used in treatment of myasthenia gravis (Coulter, 1994). Usage of krait venom to treat different diseases was mentioned in homeopathy and Chinese medicine. Very few reports regarding therapeutic potential of two types of Indian krait *B. fasciatus* and *B. caeruleus* venom have been available so far.



Fig 1: *Bungarus caeruleus* in India (Source: https://www.inaturalist.org/taxa/96224-Bungarus-caeruleus/browse_photos)

Source: www.iucn.org.



Fig 2: *Bungarus fasciatus* (Source: <https://www.indianreptiles.org/sp/420/Bungarus-fasciatus>)

An antivenom antibody was raised in chicken against *B. caeruleus* and *Naja naja* venom was experimentally used *in vivo* and *in vitro* experiments to neutralize venom induced toxicities. This specific antibody (IgY) had molecular weight of 180 kDa and able to neutralize venom induced lethal effect on Swiss albino mice, phospholipase A₂ activity, paw edema activity. In procoagulant neutralizing experiment with IgY, clot formation was not observed. Cathelicidin-BF a cathelicidin like antimicrobial peptide was purified from *B. fasciatus* through gel filtration and RP-HPLC was tested against forty (40) microorganism strains. Cathelicidin-BF was mostly potent against Gram negative bacteria like *E. coli*, clinically isolated strain *Salmonella typhi* and also effective against fungi like *C. albicans*, saprophytic fungus (*A. terreus*). Clinically used antibiotics like ampicillin, benzylpenicillin possessed no effect on any of these strains (Wang et al., 2008). In another study it had been found that Cathelicidin-BF killed *Propionibacterium acnes*, *Staphylococcus epidermidis* responsible pathogen for acne vulgaris. It inhibited *P. acne* induced O₂^{•-} production, pro inflammatory cytokines production *in vitro*. In mice model *P. acne* induced ear swelling, granulomatus inflammation was successfully restored after

Cathelicidin-BF treatment (Wang et al., 2011). Therefore, Cathelicidin-BF has potency for therapeutic use against resistive strains of different microorganisms as well as against acne vulgaris. A L-amino acid oxidase from *B. fasciatus* purified through chromatographic procedure having molecular weight of 70 kDa, showed cytotoxic effect on A549 (adenocarcinomic human alveolar basal epithelial cells) cell line after 12 hrs treatment (Wei et al., 2009). A neurotoxin purified from *B. caeruleus* caused blockade in isolated frog rectus abdominis muscle preparation and might be used as a therapeutic agent in the treatment of neuromuscular disorder (Mirajkar et al., 2006).

A novel group IAs PLA₂ named BFPA isolated from *B. fasciatus* snake venom showed potent bactericidal activity against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria. This BFPA also increased Ca²⁺ induced clotting time of platelet poor plasma i.e showed anticoagulant activity (Xu et al., 2007). This can provide a drug development evidence against thrombosis and related cardiovascular diseases.

An anti PLA₂ rabbit antiserum was produced against a toxic PLA₂ (BF-38) of *B. fasciatus* venom after hyperimmunization of rabbit. This

anti PLA₂ rabbit antiserum along with 2- hydroxy-4-methoxy benzoic acid (a herbal origin venom antagonist) neutralize venom induced toxicities like PLA₂ activity, paw edema, plasma recalcification and cardiotoxicity which is comparable with commercial polyvalent anti snake venom antisera. So, in case of banded krait envenomation the combination therapy of anti PLA₂ rabbit antiserum with herbal antagonist might produce better snakebite management in rural areas (Gomes et al., 2012). The krait venoms/toxins may also help in developing newer antidotes for snakebite victims.

CONCLUSION

Although limited scientific reports are available on Indian krait venoms and their purified toxins, they do imply towards therapeutic potentials against neurological disorders, bacterial, parasitic infections, cancer and several other diseases. Further detailed study with krait venoms may decipher not only new intimations towards developing novel life saving drugs and diagnostic reagents in the near future but also elucidating as a biological tool.

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“Zebrafish (*Danio rerio*)” is a New Trend in Biomedical Research as a Model Organism

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ABSTRACT

Animals as model organism, the silent sentinels stand watch over the environmental health of the earth. These are non human animal species which can be utilized to understand certain biological processes and to obtain information on which can be provided an insight into the functioning of other organisms. In the middle of the model organisms, the zebra-fish (*Danio rerio*) is one of the best leading model to study developmental biology, cancer, toxicology, drug discovery, and molecular genetics. Furthermore, the zebrafish is increasingly used as a genetic model organism for aquaculture species and in toxicogenomics as well as to generate zebrafish diseases models for application in human bio-medicines. This minute fish is a versatile model organism for many fields of research because of its easy maintenance, breeding and transparent body in the course of early development Gore et al. (2012).

Keywords: Zebrafish, Model Organism, Cancer, Biological Research

INTRODUCTION

The “Zebrafish model” has been widely used in both animal and human health research, and more recently aquaculture too. In spite of rodents being the most widely used research model in the world. In recent decade the use of the Zebrafish (*Danio rerio*) model has exponentially increased among the scientific community.

The use of Zebrafish as a model organism was pioneered at the University of Oregon, USA by George Streisenger in 1970 because it was simpler Over mouse and easy to manipulate genetically But this species as model organism got impetus from the 1990’s when it was used to developed two large genetic mutants, one by Nobel Prize winner Christiane Nusslein - Volhand in Tubingen, Germany, the other by Wolfgang Driever and Mark Fishman in Boston, USA. According to Kanungo et al. (2014) a lot of physiological and genetic similarities are found

between Zebrafish and humans, including the digestive tract, brain, vasculature, musculature and innate immune system Also 70 percent of human disease genes have functional similarities with those of Zebrafish.

Range:

The Zebrafish is native to fresh water habitats in South Asia where it is found in India, Bangladesh, Nepal and Bhutan.

Habitat:

It is typically inhabit moderately flowing to stagnant clear water of quite shallow depth in streams, canals, ditches, ponds, rice paddies etc.

Description:

Zebrafish named for five uniform pigmented, horizontal blue stripes on side of the body, which are reminiscent of a Zebra’s stripes and which extend to the end of the caudal fin Salient distinguishable structures present between male and female. The Zebrafish reach

Taxonomic Rank	Classification
Kingdom	Animalia
Phylum	Teleostei
Sub-Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	Danio
Species	Danio rerio

up 4 to 5 cm in length.

Reproduction:

Female spawns every 2 to 3 days, breed all year round external fertilization

Feeding:

Omnivorous

Zebrafish As A Biomedical model Why replace mammal model?

- The mammal models (rodents) can be easily stressed, so some human behaviors cannot be fully explored under these conditions.
- Large space for creation.
- Collecting mouse embryos requires sacrificing the female.

Salient features of Zebrafish as a biomedical model:

- Optically translucent embryos.
- Rapid hatching of eggs.
- The adult fish attains sexual maturity very quickly, with a generation time of about 10 weeks.
- They are cheaper to maintain than mice.
- Amenable for molecular and genetic analysis.
- Zebrafish have the unique ability to repair heart muscle.
- They share 70% of genes with humans. Kalueff_et_al. (2014)

3. Methods and Techniques Used in Zebrafish Research

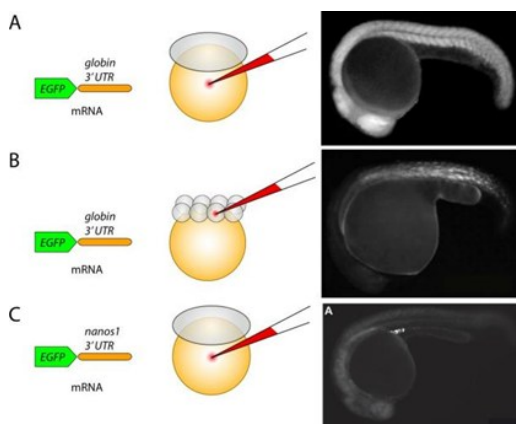


Figure 1: Injection into zebrafish embryos at the early stages of development.

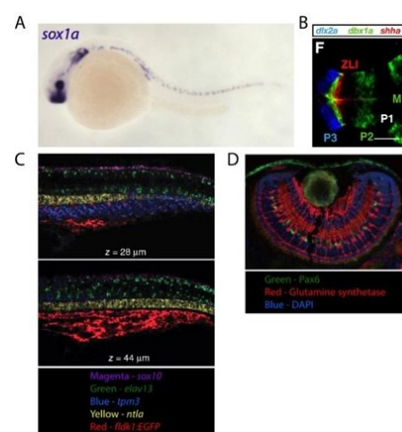


Figure 2: Gene expression and protein visualization.

Use of Zebrafish in developmental biology:

The zebrafish model has been used to see the development of various systems or processes as follows

a) Development of the ENS:

This model species has been made to study the development of the enteric nervous system (ENS) in recent time Guyon et al. (2007). Like all Other vertebrates, the enteric nervous system of Zebrafish is also derived from neural crest, but differs potentially from amniotes where in the enteric nervous system is derived from both the vagal and sacral crests, while in the Case of Zebrafish, it is derived from the vagal crest only.

b) Angiogenesis:

The Zebrafish model has been widely used in the study of angiogenesis and regeneration. Angio- genesis is the process through which new blood vessels original from preexisting Vascular structure which is essential to maintain healthy physiological and pathological conditions.

The Zebrafish exhibits remarkable capacity of regeneration even in adult stages. Due to its simple and fine architecture and relative transparency Caudal fin especially provide an ideal tissue for vascular regeneration studies

The zebrafish as an alternative model for angiogenesis and regeneration studies provide the rele- vance of in vivo assays with simplicity and Versatility of in vitro assays.

4. Zebrafish as a cancer model system

Clinically and pathologically this dreaded disease is found almost exclusively in all vertebrates, from fish to humans. Being a vertebrate the Zebeafish is an ideal model to study cancer, though humans and fishes are separated from their common ancestry but biology of the cancer in both groups of organism is not different. Briggs (2002)

4.1 Carcinogen effects and development of cancer

Fishes are exposed to numerous waterborne carcinogens in the wild which lead to the growth of a variety of benign as

well as malignant tumors in teleosts, and these tumors have similar histology as in humans. Selecting Zebrafish for modeling Cancer studies has many advantages. Extremely conserved cancer pathway can be screened genetically using Zebrafish. Essentially cancer is a disease of adults, but through mutagenesis screens, cell cycle phenotype could be examined in quickly developing transparent embryos of the Zebrafish.

Tumorigeneris

Tumorigeneris is a multi steps process induced by chemical carcinogen, with cumulation of both epi- genetic aberrations and mutations in regulatory regions of genes and disorder of signaling pathways. A variety of fishes to put into action as model to study tumors induced by environmental carcinogen. Among all the Zebrafish proved best for studying embryogenesis, organogenesis and impact of envi- ronmental carcinogen for the growth of cancer. chemically induced tumors in Zebrafish and humans are histopathologically alike, and orthologous oncogenes and tumor suppressor genes (TSGs) have been recognized in fishes and humans. Weinstein (2002) Hepatic gene expression in humans and Zebrafish have has disclosed conservation of gene expression profiles at different stage of tumor aggressiveness between these two phylogenetically outlying species.

Xenotransplantation

It represents another new way to induce tumor in Zebrafish. The significant feature of Xenotransplanta- tion is that tumor cells can be stained or marked using fluorescent stain that differentiate transplanted cells from normal Cells and helps in clear observation of developmental process of the tumor. A number of other types of tumor, such as pancreatic cancer, lung cancer, ovarian carcinoma, breast cancer, prostate cancer, retinoblastoma leukemia etc, have also been transplanted in the Zebrafish.

Skin cancer

Zebrafish is a influential in vivo tool to study pathologies and treatment for skin cancer

(melanoma and SCC). It can be used to study melanoma development, progression, drug screening, and treatment. The Zebrafish model has been utilized recently to recognize the Key molecules which are responsible for the development of cutaneous squamous cell carcinoma (CSCC) and head and neck squamous cell carcinoma well as for SCC target therapies. Bootorabi et al. (2017)

Tumor metastasis

Metastasis, is a multi step and complex process in which tumor cell impale in the vascular system and spread deep in parenchymatous tissues. Because of many noteworthy disadvantages in the previous studies using in Vivo mouse model, the metastasis process can not be abstracted probably, but Zebrafish cancer model has overpower the drawback of previous models and has shown extraordinary strengths.

Toxicology and drug discovery

Because of numerous advantages, the Zebrafish has recently appeared as a prominent model for toxicological studies and drug discovery. The effects of drugs on growth and development can be investigate visually through length and shape of the Zebrafish body as well as morphology of internal organs such as the liver, brain, cardiovascular system, intestine, pancreas, kidney, notochord etc.

Zebrafish embryos are rused as predictive model to assess the toxicity in mammals. The lethal concentration (LC50) of divergent chemicals has been determined in embryos of Zebrafish has been compared with the mammalian LC50 and it has been found that median lethal dose of Zebrafish is lower than mammals. The effects of drugs on specific organs have also been studied, and it has been found that organ toxicity is similar in both Zebrafish and mammals. The drugs that utilized to evaluate the organ toxicity were gentamicin, cisplatin, quinine, vinblastine, doxorubicin, neomycin, cyclosporin A, caffeine etc. Pelegri (2002)

Human disease and Zebrafish

Most of the tissues and organs found in humans and Zebrafish are the identical except lungs and prostate and mammary glands. The cloning of mutated genes screened for specific phenotypes in Zebrafish has resemblance in humans and thus serves as model for human disease and to study underlying mechanisms the first human disease identified using Zebrafish was a blood disorder relate to specific defect in hemoglobin production through ALAS2 mutated gene. Many other mutants which show phenotypic comparability to human disease have been screened and identified. These include neurological disorders, hematological disorder, Cardiovascular diseases, muscle disease and Cancers, Parkinson's disease, anxiety and post-traumatic stress disorder. Guyon et al. (2007)

Zebrafish as a model organism for aquaculture species

Among the different fish species of interest to aquaculture, Zebrafish is genetically more tractable. The Zebrafish model is used commercially in a lot of areas of aquaculture such as in the identification of genes involved in the development of the muscles, bones and fats, the metabolism of nutrients, disease and stress physiology and also behavioural traits. Bailone et al. (2020)

Future Prospects (Conclusion)

- Zebrafish have the rare ability to repair their own tissue, including spinal cord, retina, and heart—understanding this could lead to the development of new human treatments.
- Zebrafish are becoming an increasingly successful model to understand Alzheimer's disease, with future scope for investigation in neurodevelopment and neurodegeneration, which promises exciting research opportunities in the future.
- The speed and innovation advantages of zebrafish offer future opportunities to expand the positive impact of our discipline and help to meet the challenges facing 21st-century societies.

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A study on present status of *Bos frontalis* (Mithun) found in Arunachal Pradesh, India

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ABSTRACT

Mithun is a semi domesticated animal species of cattle which has a high value among the tribal communities of Arunachal Pradesh. This wonderful species is facing threats of extinction due to overexploitation for various purposes. This paper focuses on their cultural, economical and ecological significances and the conservational efforts to protect this species. A questionnaire has been prepared and personal interviews were taken of local people who possess Mithuns. The study finds the various significances of Mithun and conservation efforts by the government and the local communities to protect the species.

Keywords: Mithun, indigenous, tribal communities, ethnic tribes, ecological significance

INTRODUCTION

Mithun (*Bos frontalis*) is a unique species of cattle that is majorly found in the regions of North Eastern states and Himalayas. It has a deep impact in the social, cultural and indigenous believes of the tribal and indigenous communities residing in those areas. This species has several uses, it is majorly used for the meat, as it has a much higher value in the local markets as compared to any other sources of meat available. This species also plays a crucial role in mountain agriculture, providing food security to the local people. In India, Mithun is mostly found in Arunachal Pradesh (state animal), Manipur, Nagaland and Mizoram. The population of Mithun is approx. 2,98,264 out of which 2,49,000 are found solely in Arunachal Pradesh (livestock census of india,2012). In Arunachal Pradesh, the highest population of Mithun is present at lower subansari district with 31,262 & lowest in upper

dibang valley with 1735 [Apum et.al, 2020]. The tribal communities of Arunachal pradesh have their own religious and cultural beliefs regarding Mithuns. Possessing Mithuns is considered to be a sign of prosperity for them. But however, in recent years, Mithuns are facing a threat to extinction due to mass harvesting for its meat. Mithuns are harvested in huge numbers during marriages which is one of the leading causes of decline in their population. It is also harvested for the horns which is a traditional decorative item in tribal households. All these practices, eventually leads to decrease in population of Mithun. But even so, there are no such adequate number of policies that can play a part in the conservation, probably due to its small population and presence limited to certain parts of India. The exploitation and destruction of their habitat (generally the forests) is one of the major factors in reducing and hampering their population.

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METHODS AND METHODOLOGY

STUDY AREA: The study area comprises of one of the districts of Arunachal Pradesh i.e. East Kameng, which is inhabited mostly by Nyishi tribe. The major study site was Seppa (district headquarter) and its nearby villages. East coming has a total geographical area of approx. 4,134 km² and located in between 27.15°N to 28.25°N latitude & 92.15°E to 93.05°E longitude. Kameng river is the major river that flows in the particular study site. The total population of the whole area was 78,413 as per census of India 2011. This area was particularly chosen due to the adequate vegetation and suitable weather condition for to the survival of the species.

DATA COLLECTION

The data has been collected from both the primary and secondary sources:

- Primary Source.** The data was mostly Collected Primarily by carrying out a detailed survey by filling up the questionnaire & interviews. About 40 household of 3 villages who either possess Mithun or take care of them during the period of survey (Jan 7-17) were interviewed to derive the information such as (total

population, life expectancy, local name, cultural & economical values, breeding seasons etc) of Mithun. Cameras and Phone were used to collect and store the information of the survey.

- Secondary source:** Some of the information was also collected from library, books, journal, research paper, newspapers and other various means. This includes the ecological and economical value of the species

SURVEY

The various photographs and information of the particular species has been collected through visiting various places of the study site. The photograph has been collected from the forests and hills of the study site. The photographs show about the nature and preferred vegetation of the species. This particular species prefer to live in solitude and is a herbivorous in nature. They are generally found near the water sources, which majorly is the river and the waterfalls. Detail survey has been done in order to fill up the questions about the species. About 40 families of three villages (both adult males and females) were interviewed to collect the information regarding



Source: mapsofindia.com)

Fig.1: Map showing study site

the habitats and behavior of Mithun. Cameras and phones have been used to collect and store the visual information of the survey.

MITHUN

Mithun, also known as the Gayal, is a large domestic animal distributed in Northeast India, Bangladesh, Myanmar and China. Mithuns are larger, well-built animals typically larger than domestic cattle. They are reddish brown to blackish brown in color due to the presence of hair all over the body except their legs which is mostly covered with white hairs or stockings. It has a long tail. Adult males are larger, bigger in size and have more muscles as compared to female. Adult male can weigh up to 400 to 600 kg, while female weigh up to 300 to 400 kg. They have broad and flat head. Both the sexes possess long, thick,

pointed and curve horn on the head region which is about 0.5 to 1m in length. Male horn is generally larger than the females. They have strong and well-developed legs which helps them to walk on hilly and mountainous region and their hooves provide grip and stability to walk on the sloppy areas on the mountains. Their natural habitat is the North East India and the Himalayan regions of Indian subcontinent. The North Eastern and Himalayan regions provide optimal habitat for Mithuns. These areas are covered with dense forests, mountainous and hilly regions which is suitable for their survival. Mithuns live in humid subtropical and temperate climates. Forest vegetation is the primary source of food for the Mithun. The availability of food source like bamboo, shrubs etc is crucial to provide them with food security.



Fig. 2: Surveys performed in various area



Fig 3: Mithun (*Bos frontalis*)

CULTURAL SIGNIFICANCE OF MITHUN

Mithuns (*Bos frontalis*) has a deep cultural significance in the ethnic tribes of Arunachal Pradesh. Mithun is not only a domesticated animal but also a symbol of wealth, power, status and cultural beliefs. It has a deep impact on various aspects of tribal life like rituals, social status etc. Having a Mithun in a local household of a tribal community shows their social standing within the community. Higher the numbers of Mithuns, the higher the economical status within the community. There is also a saying that a marriage is not considered as completed, until and unless the bride's family possess the animal which is offered by the groom's family. In marriages, Mithun are used as transaction for dowry which is generally given to the bride's family from the groom's family. The meat of Mithun also plays a crucial role in marriage ceremony. Mithun plays a central role in various cultural and religious rituals. It is also referred as the descendent of the sun and sacrificed in various festivals as an offering to the Gods or deities. It is believed that these sacrifices bring prosperity and protection to one's household or community. It has been informed by local people of the area that to domesticate Mithun, they used to feed salt to the young babies of the Mithun at an early stage of their life. This particular cultural ritual has been passed on from generation to generation within the tribe community.

ECONOMICAL SIGNIFICANCE OF MITHUN

Mithun has various economical significance which helps in the survival of ethnic Tribes of Arunachal Pradesh. This domesticated species contributes a lot to their economy in various ways. Although, Mithun is not considered as a farm animal, but even though it has a significant use in field work of some areas. The manure obtained from Mithun is a vital resource for traditional farming practices in the hilly areas. It is used as organic and natural fertilizers, which enhances soil fertility and provides better crop production. Mithun are a valuable asset in the livestock market. They are bought and sold for

significant amount of money (75,000 to 1,25,000) depending upon health, sex, weight and other qualities which provides a crucial source of income for the tribal household. Compared to other cattle's, the quality of meat from the Mithun are considered to be superior as it contains more nutrition and protein than an average cattle.

Mithun are also considered as a huge asset or capital. It is often used in barter trades and in solving social and legal disputes within the communities. Mithuns are also used as collateral or as a mode of security while taking loans from local community lending groups which helps in urgent financial issues.

ECOLOGICAL SIGNIFICANCE OF MITHUN

Mithun (*Bos frontalis*) holds some ecological significances in Arunachal Pradesh. Since it is a semi domesticated animal, it is closely related with the natural habitat.

But even though they are not much information about there ecological significance. This species is generally located closer to a fresh water source and the presence of this species shows the richness of the diversity of the particular place. They generally live in dense forest region, rarely coming or towards crowded human civilization. Their presence in a particular area has a great influence on the population of other herbivores and predators which contributes towards the ecological balance. Due to their semi domesticated in nature, this animal plays a crucial role in conserving their natural habitat.

CONSERVATIONAL EFFORTS:

In recent times, Mithun has been categorized as vulnerable species according to IUCN red list data (<https://www.iucnredlist.org/>). Conservational efforts are important due to their cultural and economical significances. Habitat conservation is one of the most basic setup required for Mithun conservation. Protecting the forest areas in which Mithun generally grazes is required to ensure their food security. Additionally, harvesting of Mithun for meat and other purposes is now under legal guidance in

various areas which prevents mass harvesting of this species. Government of Arunachal Pradesh also provides subsidies and facilities for regular veterinary checkup and vaccination to prevent any kind of diseases. Veterinary services are also established in various remote areas to provide essential medical checkup and care to Mithun.

The communities also needs to play role in Mithun conservation by apply indigenous knowledge and practices in Mithun management. Also creating awareness and educating other local people/communities about sustainable harvesting and usage is very important in conservation of Mithun.

DISCUSSION

Mithun is an uncommon bovine species of cattle found in the regions of North Eastern states and Himalayas. They have a huge impact on the social, cultural and economical lives of the indigenous and tribal people of the area. But due to overexploitation of such wonderful creature for its meat, horns, leather etc, it is now facing a threat of extinction. The local people should come forward to protect and conserve these beautiful species from extinction.

We should aware local people to look after the Mithun to improve the healthcare and conservation of the species. Organizing various awareness campaign will play an important role in their conservation. They are considered as the umbrella species as they are naturally selected for conservation related decisions of the habitat. Protecting and conserving this species indirectly benefits various others species, thus contributing in the formation and development of an ecological community.

CONCLUSION

Mithun is an important semi domesticated animals species of hilly and mountaineous regions of India, specially Arunachal Pradesh. It plays a crucial role in cultural, economical and ecological significance of ethnic tribes of Arunachal Pradesh. It is a herbivore animal which grazes on various areas and is mostly found near fresh water sources but due to shrinkage of forests caused by various man made activities, the Mithun population began

to decline and is now limited to certain areas. There are many ways which can enhance Mithun's productivity and tribal's livelihood. The Government of Arunachal Pradesh is also keen on adding new schemes and subsidies which can contribute towards Mithun's health and lifestyle. But even though, there is lack of such schemes and poor implementation of available schemes, leading this wonderful species towards extinction. Unless and until, the government and the local people start emphasizing more on conservation efforts, the population will reduce to such an extent that this uncommon and beautiful species will go extinct.

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A Critical Exploration of Selected American Short Stories: Themes, Techniques, and Cultural Contexts

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ABSTRACT

This research paper critically examines how four iconic American short stories—Washington Irving’s *The Devil and Tom Walker*, Flannery O’Connor’s *The Lame Shall Enter First*, Edgar Allan Poe’s *Ligeia*, and Ernest Hemingway’s *Indian Camp*—articulate evolving cultural values concerning death, morality, and human consciousness. Through a comparative literary analysis, the study explores how each author uses distinctive narrative strategies, symbolism, and thematic preoccupations to reflect the social, theological, and psychological anxieties of their respective periods. Employing a multidisciplinary theoretical framework—including narratology, psychoanalysis, feminist critique, ethical criticism, and posthumanism—the paper demonstrates how literary form and ideology intersect in shaping the reader’s engagement with mortality. Irving’s allegorical satire critiques capitalist greed within a Puritan worldview, while O’Connor interrogates modern secularism through Catholic theology and Southern Gothic conventions. Poe’s gothic romanticism explores the uncanny and the limits of reason, and Hemingway’s minimalist realism presents death as a silent existential rupture. By analyzing these stories in parallel, the paper reveals a significant trajectory in American literature—from moral didacticism to existential ambiguity—underscoring how death serves as a cultural lens for understanding shifting American ideals and philosophical concerns. The study concludes that American short fiction remains a vital literary form for confronting mortality, not just as an event, but as a site of ethical, emotional, and ideological struggle.

Keywords: American short fiction, death and morality, narrative strategies, cultural values, literary theory

INTRODUCTION

The American short story, as a literary form, has long served as a mirror to the nation’s shifting moral, cultural, and existential landscapes. By condensing complex themes into brief yet powerful narratives, short fiction offers a concentrated lens through which evolving American values—particularly regarding death, morality, and human consciousness—are explored. This paper takes four landmark stories by

Washington Irving, Flannery O’Connor, Edgar Allan Poe, and Ernest Hemingway, each representing a distinct historical moment and philosophical orientation. From the allegorical condemnation of greed in Irving’s early American satire to the existential silences of Hemingway’s modernist realism, these texts chart a trajectory in literary consciousness that reflects broader socio-cultural transitions. Drawing on diverse theoretical frameworks—including

psychoanalysis, narratology, semiotics, feminism, and posthumanism—this study interrogates how narrative structure, symbolic imagery, and ideological positioning shape each story's engagement with mortality. In doing so, it reveals not only the richness of American short fiction but also its persistent relevance as a cultural site for negotiating the boundaries between life and death, faith and reason, and self and society.

The selected stories span the 19th and 20th centuries, offering a wide-ranging view of American literary sensibilities and social contexts. Through close readings, this study analyzes how these narratives engage with enduring human concerns such as greed, morality, redemption, death, and existential anxiety. In doing so, it interrogates the ways in which authors use symbolism, irony, narrative voice, and setting to construct meaning and provoke reflection.

Washington Irving's *The Devil and Tom Walker* functions as a powerful cautionary tale about greed, moral hypocrisy, and the dangers of materialism in early American society. The story follows Tom Walker, described as "a meagre, miserly fellow" who lives in a "forlorn-looking house that stood alone and had an air of starvation." (Irving, 1824) One day, Tom meets the devil—"a great black man seated directly opposite him, on the stump of a tree... swarthy and dingy, and begrimed with soot, as if he had been accustomed to toil among fires and forges" (Irving, 1824)—and learns of buried treasure hidden by the pirate Kidd. The devil offers to make Tom rich, but "they were to be had only on certain conditions," (Irving, 1824) which, though never fully disclosed, imply the forfeiting of Tom's soul. After his wife's mysterious disappearance during her own attempt to make a deal, Tom accepts the devil's bargain and becomes a usurer. He opens a broker's shop in Boston and soon is exacting "good pay and security" from the desperate. He proudly declares, "I'll drive them to the devil," (Irving, 1824), demonstrating the extent of his moral decay. Though he later becomes a zealous church-goer—"He prayed loudly and

strenuously, as if heaven were to be taken by force of lungs" (Irving, 1824)—this religious fervour is clearly performative. His fate is sealed when, in a fit of arrogance, he exclaims, "'The devil take me," said he, "if I have made a farthing!"' (Irving, 1824). Immediately, "a black man was holding a black horse," and tells him, "Tom, you're come for." (Irving, 1824). He is whisked away during a thunderstorm and never seen again. Later, his wealth is found to have vanished: "his iron chest was filled with chips and shavings," and "his great house took fire and was burned to the ground." (Irving, 1824) his iron chest was filled with chips and shavings Through these events, Irving critiques not only individual vice but also the spiritual emptiness and social corruption enabled by early American capitalism, using irony, allegory, and dark satire to deliver his ethical vision.

In *The Lame Shall Enter First*, Flannery O'Connor explores the tension between rational morality and spiritual redemption through the character of Sheppard, a widowed social worker guided by secular humanism. His misguided efforts to rehabilitate Rufus Johnson, a troubled and deeply religious youth, reflect the conflict between modern social reform and traditional spiritual grace. Sheppard believes that "If you stop thinking about yourself and think what you can do for somebody else," Sheppard said, "then you'll stop missing your mother." (O'Connor, 1965), a statement emblematic of his reductive moral logic that dismisses the depth of his son's grief. O'Connor, known for her Catholic vision, deliberately contrasts Sheppard's logical worldview with Rufus's rigid, fundamentalist faith to critique modern liberalism's failure to acknowledge the mystery of spiritual suffering. Rufus declares with conviction "Satan," he said. "He has me in his power." (O'Connor, 1965), resisting Sheppard's psychological explanations and instead clinging to his vision of divine judgment and damnation. As critics have noted, Sheppard's refusal to confront his emotional limitations results in alienation from both Rufus

and his son Norton (Giannone, 2000). When Norton asks, “Is she there, burning up?” referring to his deceased mother, Sheppard’s replies, reveals his incapacity to offer emotional or spiritual consolation. The story employs biblical allusion, irony, and Southern Gothic motifs to underline the moral complexity of compassion. The title itself, referencing Luke 14:21, ironically contrasts Sheppard’s material charity with his spiritual emptiness. Ultimately, O’Connor dramatizes a theological paradox: efforts to do good without spiritual humility may lead to greater harm. As Kirk (2009) argues that humanitarianism divorced from humility becomes a destructive moral pride and Sheppard exemplifies this in his belief that rational goodwill is enough to redeem others. This hubris is shattered when Rufus tells Sheppard, “Nobody can save me but Jesus” (O’Connor, 1965), underscoring the insufficiency of secular altruism in the absence of grace.

While O’Connor emphasizes the dangers of secular rationalism in the face of spiritual mystery, Poe shifts the focus inward, illustrating a similar arrogance through the narrator’s refusal to accept the finality of death.

Poe’s *Ligeia* is a gothic tale that interrogates Romantic ideals of eternal love and the human will’s defiance of death. The story, narrated by an unreliable protagonist, oscillates between memory and hallucination, making it a prime example of what critics call Poe’s “narrative instability” (Silverman, 1991). This instability is most evident in the climactic moment when the narrator claims that the dying Rowena transforms into the resurrected Ligeia—a moment shrouded in ambiguity and obsession. The narrator cries: “these are the full, and the black, and the wild eyes — of my lost love — of the lady — of the LADY LIGEIA” (Poe, 1838), blurring the boundary between reality and delusion. According to Kennedy (1987), Ligeia embodies Poe’s ideal woman—intellectually superior, spiritually dominant, and ethereal—an archetype that recurs throughout his fiction. The narrator himself remarks, “I have spoken of the learning of Ligeia:

it was immense — such as I have never known in woman” (Poe, 1838), and he attributes to her a seemingly supernatural will, quoting Glanvill’s maxim: “Man doth not yield him to the angels, nor unto death utterly, save only through the weakness of his feeble will” (Poe, 1838). The narrator’s descent into madness mirrors broader 19th-century anxieties about reason, science, and mortality. His mental state is further clouded by addiction: “I had become a bounden slave in the trammels of opium...” (Poe, 1838), placing him in a hallucinatory space where perception and reality become indistinguishable. Poe’s lush, arcane language and claustrophobic setting heighten the psychological intensity, reflecting the Romantic fascination with the sublime and the grotesque (Buranelli, 1961). The narrator describes the bridal chamber in which Rowena dies as “one chamber, everaccursed” with moving tapestries and funereal drapery that “writhed in and out of them, as if endued with a serpent vitality,” (Poe, 1838), emphasizing horror and disorientation. As such, *Ligeia* becomes not merely a love story, but a meditation on obsessive idealization and the unreliability of perception. Ligeia, to the narrator, was “the one only and supremely beloved” (Poe, 1838), and her haunting return—real or imagined—suggests that love, like death, may be ruled more by the mind’s distortions than by reality itself.

In contrast to Poe’s elaborate style and metaphysical obsession, Hemingway strips language to its essence to convey existential themes through minimalism and restraint. In *Indian Camp*, Hemingway crafts a powerful coming-of-age narrative using his “iceberg theory” to embed deep emotional meaning beneath a surface of simple prose. The narrative’s restrained style conceals profound emotional and existential themes, particularly through its minimalist depiction of trauma and transformation. Young Nick Adams’s exposure to both childbirth and suicide initiates him into adult realities of suffering, gender roles, and mortality. Early in the story, Nick’s father informs him: “This lady is

going to have a baby, Nick,” and then explains, “What she is going through is called being in labor. The baby wants to be born and she wants it to be born. All her muscles are trying to get the baby born. That is what is happening when she screams.” (Hemingway, 1924). Nick’s immediate response— “Oh, Daddy, can’t you give her something to make her stop screaming?” (Hemingway, 1924) —reveals his emotional sensitivity, which contrasts sharply with his father’s clinical detachment: “I don’t hear them because they are not important.” (Hemingway, 1924). Scholars have pointed out that the story critiques patriarchal control and emotional repression, especially through the figure of Nick’s father, whose scientific authority overrides empathy (Modelmog, 2002). This is exemplified during the Caesarean delivery, when the woman is forcibly held down: “Uncle George and three Indian men held the woman still,” (Hemingway, 1924) and later, she is completely unconscious of her condition— “She did not know what had become of the baby or anything.” (Hemingway, 1924) The tension between cultural dominance and Indigenous suffering is underscored by the passive, voiceless Native characters—a reflection of colonial power dynamics in early 20th-century America. The Native woman’s suffering is endured in silence, while her husband lies above, unnoticed until he is discovered to have “cut his throat from ear to ear.” His voiceless suicide stands as a grim metaphor for colonial erasure and despair. The white doctor’s intervention—bringing medicine and surgery but no emotional engagement—further reflects imperial authority disguised as benevolence. The symbolic use of the lake crossing— “Nick lay back with his father’s arm around him. It was cold on the water.” (Hemingway, 1924)—and the stark juxtaposition of birth and death encapsulate a moment of existential rupture for Nick, marking the beginning of his philosophical reckoning (Benson, 1975). The boat ride becomes a

modernist threshold, moving Nick from childhood innocence into traumatic awareness. As the story closes, Nick’s belief that “he felt quite sure that he would never die” reflects a child’s effort to process trauma through denial. This final line, delivered in Hemingway’s understated style, conveys both psychological defense and the deep emotional impression left by the morning’s events. It is a stark contrast to Poe’s Gothic narrators, whose denial of death leads to madness; in Hemingway’s world, such denial becomes quiet endurance rather than dramatic collapse—a mark of his modernist restraint.

Death, a central theme in literature, manifests diversely across these stories, shaped by the authors’ cultural and historical milieus.

In Irving’s *The Devil and Tom Walker*, death operates as divine retribution. Set in the 18th century and steeped in Puritan ethics, the story critiques moral decay and greed. Irving uses allegory and satire to expose hypocrisy, particularly through Tom’s false religiosity. As the narrator notes, Tom “was as rigid in religious as in money matters,” attending church not from piety but self-interest. The eerie setting of the “thickly grown” forest, where “no birds sang,” symbolizes moral corruption and the lurking presence of evil. Old Scratch himself serves as a personification of temptation and judgment. When Tom rashly cries, “The devil take me,” said he, “if I have made a farthing!” the literal fulfilment of this curse underscores the story’s didactic tone and its alignment with the Romantic period’s moralism (Levine, 2007).

In contrast, Flannery O’Connor’s *The Lame Shall Enter First* engages death through a theological lens. Her Catholic worldview informs a narrative in which grace and redemption are central. The protagonist Sheppard, a secular rationalist, attempts to redeem Rufus Johnson through logic and charity, but fails to grasp the boy’s spiritual anguish. Rufus, convinced of his own damnation, tells Sheppard, “I’m going to hell anyway.” This chilling assertion reflects his internalization of sin and his longing for spiritual

truth, which Sheppard cannot comprehend. O'Connor's use of Southern Gothic conventions—such as grotesque characters and moral extremity—frames death not as an end, but as a spiritual reckoning (Jacob, 2019). In the final scene, Sheppard realizes the cost of his intellectual arrogance too late, underscoring O'Connor's critique of humanism divorced from faith.

Unlike O'Connor's use of death as spiritual revelation, Poe's portrayal emphasizes psychological obsession and metaphysical ambiguity. In *Ligeia*, death is not a moral endpoint but a boundary that the human will seeks to transcend. The narrator, whose reliability is undermined throughout the text, becomes obsessed with the idea that Ligeia's can overcome death. His claim that "Man doth not yield himself to the angels, nor unto death utterly, save only through the weakness of his feeble will." (Poe, 1838). encapsulates the story's metaphysical challenge. The moment when Rowena's corpse seemingly transforms into Ligeia remains ambiguous—an opium-induced hallucination, a supernatural event, or a projection of madness. Poe's ornate diction and Gothic setting intensify the story's dreamlike tone, while the recurring motif of Ligeia's "large and luminous" eyes symbolizes both spiritual power and the haunting gaze of the dead (Vuković, 2019).

In contrast to Poe's elaborate psychological interiority, Hemingway's *Indian Camp* presents death as a stark, natural phenomenon, devoid of mysticism. Set in a rural 1920s American landscape, the story follows young Nick Adams as he witnesses a Caesarean birth and the Native husband's subsequent suicide. The juxtaposition of birth and death is conveyed without melodrama but through Hemingway's restrained prose. "The Indian lay with his face toward the wall. His throat had been cut from ear to ear. The blood had flowed down into a pool where his body sagged the bunk," (Poe, 1838) Hemingway writes—direct, clinical, and emotionally stripped. This detachment, mirrored in Nick's father's professional demeanour,

exemplifies Hemingway's "iceberg theory," where emotional weight lies beneath plain surface description. The story's closing line—"he felt quite sure that he would never die"—captures a child's effort to rationalize trauma, presenting death not as a metaphysical event but as a deeply psychological rupture (Greaney, 2006; Forrest, 2020).

The four stories, when viewed comparatively, reveal shifting American attitudes toward death. Irving and O'Connor present death as a moment of moral or spiritual culmination, each grounded in specific religious worldviews—Protestant and Catholic, respectively. Poe and Hemingway, however, explore death through subjective consciousness: Poe through gothic terror and metaphysical yearning; Hemingway through existential confrontation and emotional denial.

Their literary techniques likewise differ. Irving's use of allegory contrasts with O'Connor's Southern realism and biblical allusions. Poe's lyrical, ambiguous style reflects Romantic preoccupations with the sublime and the uncanny, while Hemingway's minimalist form strips death of sentimentality, reflecting modernist sensibilities.

Together, these stories underscore how American writers across two centuries have employed distinct strategies—symbolism, voice, setting, and tone—to engage with the universal theme of death. From moral retribution to metaphysical obsession, from spiritual revelation to emotional reckoning, death functions not merely as an end, but as a mirror of human values and anxieties.

II) COMPARATIVE THEMATIC ANALYSIS: DEATH, MORALITY, AND NARRATIVE FORM IN FOUR AMERICAN SHORT STORIES

This analysis examines how four canonical American short stories—Washington Irving's *The Devil and Tom Walker*, Flannery O'Connor's *The Lame Shall Enter First*, Edgar Allan Poe's *Ligeia*, and Ernest Hemingway's *Indian Camp*—navigate themes of death, morality, and human consciousness. Spanning over a century, these

narratives reflect the evolving cultural, theological, and philosophical paradigms of American society. Through divergent narrative strategies and ideological positions, each story stages the encounter with mortality in unique and culturally significant ways. Applying multiple theoretical lenses—including narratology, ethical criticism, psychoanalysis, feminist performativity, affect theory, posthumanism etc—this comparative analysis seeks to uncover the multidimensional literary engagement with death.

Irving's early 19th-century tale draws upon Puritanical motifs and folk legend to critique the moral failures embedded in capitalist society. Set in colonial New England, *The Devil and Tom Walker* functions as a cautionary allegory, exposing how greed and hypocrisy rot the moral core of society. Irving's third-person omniscient narration and satirical tone enable moral distancing that allows readers to judge Tom's hypocrisy—most notably in his sudden religiosity, praying "as if heaven were to be taken by force of lungs."

The swamp, with its "gloomy pines and hemlocks," becomes a liminal space representing societal rot, emphasized through the image of marked trees named after supposedly virtuous citizens. These images symbolize Judith Butler's theory of performativity (Butler, 1990): Tom's outward religiosity is not grounded in faith but in performative repetition—his piety, like gender or morality, is socially enacted rather than inherently possessed. Ultimately, Irving portrays salvation as unattainable for those who treat religion as spectacle. This satirical framing anticipates American anxieties about the moral cost of materialism and the commodification of virtue.

By contrast, Flannery O'Connor's *The Lame Shall Enter First* engages with theological morality from a mid-20th-century Catholic perspective. The story dramatizes the collision between modern liberal humanism and a deeply theological worldview. Sheppard, a secular social worker, believes in the redemptive power of rationalism and moral education. Rufus Johnson, a physically disabled and emotionally tormented

delinquent, clings to a literalist interpretation of Christian salvation. Their fraught relationship reveals deep moral incongruities that erupt in the narrative's tragic climax.

Martha Nussbaum's theory of ethical criticism (1990) is apt here. She argues that literature allows readers to confront moral dilemmas in emotionally textured contexts. O'Connor's fiction offers such an encounter, dramatizing the insufficiency of reason alone to comprehend or address human suffering. Sheppard's failure to empathize with his own son, while idealizing Rufus as a 'project,' reveals a deeper spiritual arrogance masked by secular benevolence.

Lauren Berlant's concept of *cruel optimism* (2011) further illuminates Sheppard's moral flaw. His belief in rational progress—what he imagines as his ability to 'fix' Rufus—functions as an attachment that hinders rather than helps. The story suggests that moral clarity may require spiritual receptiveness, not just secular logic. O'Connor's use of irony and grotesque symbolism reinforces this complexity, pushing readers to confront how ethical intentions can become destructive when unmoored from humility or grace.

Poe's *Ligeia* stands apart for its gothic intensity and psychological interiority. The narrator's obsession with the titular character, whom he describes as possessing a "radiance as of an opium-dream," reveals a disturbed mind unraveling in the wake of grief. The narrative's descent into hallucination culminates in the ambiguous resurrection of Ligeia, possibly through the body of his second wife, Rowena. The gothic bridal chamber, adorned with "arabesque figures" and violent tapestries, serves as a material manifestation of the narrator's mental instability.

Poe's use of first-person narration is an example of what Gérard Genette identifies as internal focalization (Genette, 1980). Unlike the omniscient narration of Irving or O'Connor, Poe restricts the narrative lens to the unstable consciousness of the protagonist. This creates an

interpretive ambiguity that aligns with post-structuralist theories of textual instability and unreliable narration.

Sigmund Freud's notion of the *unheimlich*, or "uncanny," is particularly useful in unpacking the story's psychological horror. The *unheimlich* refers to the return of repressed content in a distorted, familiar-yet-strange form (Freud, 1919/1955). Ligeia's return is not just eerie—it is a manifestation of the narrator's inability to accept death; his repression of loss and desire projected onto a seemingly supernatural event.

Additionally, N. Katherine Hayles' posthuman theory (1999) sheds light on the bodily transformations at play. Ligeia's potential reanimation challenges the boundary between organic life and metaphysical persistence. Her identity becomes hybrid, perhaps a projection of technological or pharmacological influence (via opium), foreshadowing later literary explorations of the posthuman condition, where body, mind, and memory become unstable constructs. Poe's gothic vision thus becomes a meditation not just on death but on the limits of consciousness and the terror of disintegration.

In sharp contrast, Hemingway's *Indian Camp* addresses death with a minimalist style that foregrounds emotional suppression and embodied trauma. The story's focus on Nick Adams, a young boy observing childbirth and suicide within hours, offers a clinical, unadorned entry point into existential rupture. The father, a doctor, performs a Caesarean section without anaesthesia, and the husband of the mother later commits suicide by slitting his throat. Nick's stunned reaction—"He felt quite sure he would never die"—is an act of denial rather than epiphany.

Hemingway's use of external focalization, as explained by Genette (1980), limits emotional access and underscores the inexpressibility of trauma. The stripped-down prose reflects what scholars call "Hemingway's iceberg theory," where meaning lies submerged beneath surface detail. This stylistic choice is not simply aesthetic—it dramatizes masculine detachment and stoic silence

as coping mechanisms.

Judith Butler's insights into gender performance are applicable here too. The doctor's stoicism, his clinical detachment, and Nick's silent witnessing are all acts within a script of patriarchal masculinity (Butler, 1990). These performances of control and emotional restraint suppress vulnerability, reinforcing socially constructed ideals of male toughness at the cost of psychological resolution.

Hayles' concept of *embodied consciousness* further deepens the reading. Though language fails to capture trauma directly, the story's focus on the physical—the birthing, the blood, the death—anchors it in the body. Nick's experience is not intellectually processed but bodily felt, gesturing toward a kind of pre-linguistic trauma encoding that aligns with posthuman theories of affect and perception.

Together, these four stories chart a progression in American literary treatments of death—from didactic moralism to existential ambiguity, from theological determinism to psychological and cultural fragmentation. Irving's satirical omniscience and religious allegory externalize moral conflict, enabling readers to view hypocrisy with ironic detachment. O'Connor, while similarly rooted in a religious worldview, internalizes conflict through ethical and spiritual tension, offering no easy redemption. Poe's gothic interiority and unreliable narration destabilize notions of identity and reality, while Hemingway's minimalist realism foregrounds bodily experience and emotional repression.

These shifts correspond to broader cultural movements: the decline of theological certainty, the rise of secular humanism, and the increasing recognition of psychological and affective complexity. The application of theoretical frameworks—from Genette's narratology to Freud's psychoanalysis, Nussbaum's ethical criticism, Butler's gender performativity, Berlant's cruel optimism, and Hayles' posthumanism—reveals how narrative form and ideological orientation work together to

shape literary meaning.

Irving's work shows how narrative distance enforces moral clarity. O'Connor collapses that distance, forcing readers into intimate proximity with ethical failure. Poe constructs a hall of mirrors—reality refracted through obsession—while Hemingway withholds commentary, leaving silence and surface detail to do the narrative labor. In each case, narrative form is inseparable from the story's moral, psychological, or philosophical stakes.

Semiotic theory, as developed by theorists like Roland Barthes and Ferdinand de Saussure, allows for a deeper interrogation of symbols and signs across these stories. In *The Devil and Tom Walker*, the swamp functions not only as a setting but as a signifier of moral and spiritual corruption. It is described as "thickly grown with great, gloomy pines and hemlocks... dark at noonday and a retreat for all the owls of the neighbourhood," creating an atmosphere of decay and menace that reflects Tom's moral descent. The decaying trees serve as particularly rich semiotic signs: "One of the great trees, fair and flourishing without, but rotten at the core," is marked with the name of Deacon Peabody, "an eminent man who had waxed wealthy by driving shrewd bargains with the Indians." This image signifies the disjunction between public virtue and private vice, as the healthy exterior masks inner moral decay. Most of the trees "were marked with the name of some great man of the colony," and all were "more or less scored by the axe," showing that social respectability masks spiritual ruin. Irving thus critiques the ideological underpinnings of early American capitalism, where wealth accumulation often coincides with ethical rot. Through these signs—swamp, trees, rot, and carved names—Irving constructs a semiotic landscape in which physical and moral corruption become inseparable, and the Devil, as symbolic master of signs, oversees the entire system.

In *Indian Camp*, Hemingway's minimalism gains depth when read semiotically. The contrast between the birthing hut and the

suicide symbolizes a duality of creation and destruction. The canoe crossing, though simple in description, acts as a symbolic passage from innocence to traumatic awareness—a rite of passage represented through spatial movement. The extinguishing of the lantern and the return at dawn semiotically signals both clarity and denial, as Nick concludes, "He felt quite sure he would never die" (Hemingway, 1987).

Poe's *Ligeia* is perhaps the richest in symbolic density. The bridal chamber is filled with Gothic motifs—arabesque tapestries, dark curtains, and ominous shadows—that serve as signs pointing to psychological decay. The character of Ligeia herself becomes a signifier of unattainable knowledge and immortal beauty, aligning with Barthes' notion of the mythologized image.

Jacques Derrida's theory of deconstruction reveals how each of these texts undermines the very binary oppositions they seem to establish—good/evil, reason/faith, life/death.

In O'Connor's *The Lambe Shall Enter First*, the apparent binary between Sheppard's rationalism and Rufus's fanaticism collapses. Sheppard's reason ultimately proves hollow and destructive, while Rufus's grotesque faith becomes a vehicle for emotional truth. The narrative deconstructs liberal humanism's confidence in empirical knowledge by exposing its failure to address emotional and spiritual suffering.

Similarly, *Ligeia* undermines distinctions between life and death. The question of whether Ligeia is resurrected or hallucinated destabilizes any firm epistemological ground. The narrator's identity also deconstructs he becomes a fragmented, unreliable voice incapable of anchoring truth—a textual mirror of Derridean *différance*.

In *The Devil and Tom Walker*, Irving appears to set up a moral allegory but subverts it through irony. The performative nature of Tom's late-life religiosity and the community's complicity in moral decay show that the text's

moral center is inherently unstable. It is not merely a battle between sin and redemption, but a critique of the very mechanisms used to judge morality.

Hemingway's *Indian Camp* is often read as an initiation narrative. Yet, the final line subverts the expected maturation: "In the early morning on the lake sitting in the stern of the boat with his father rowing; he felt quite sure that he would never die." (Hemingway, 1924). Rather than enlightenment, Nick's journey ends in denial. The text undercuts the bildungsroman structure and refuses closure, leaving only a trace of psychological disturbance.

Feminist theory adds another vital lens for analysis. In *Ligeia*, the titular character is both idealized and objectified. She exists primarily through the male narrator's gaze, raising questions about female agency and voice. Judith Butler's (1990) theory of performativity helps illuminate how Ligeia's identity is constructed through the narrator's obsessive, controlling discourse. Her resurrection is not a victory, but a monstrous return—a grotesque re-embodiment of male desire.

O'Connor's text complicates traditional gender roles. Sheppard's failure as a father and moral guide reflects a crisis in patriarchal authority. Obsessed with reason and reform, he dismisses his son's emotional needs, telling him, "You're going on eleven years old" (O'Connor, 1965), when Norton breaks down crying about his mother. His emotionally sterile approach exemplifies a masculinity that prioritizes logic over empathy. Meanwhile, Rufus's mother is absent, voiceless—a commentary on how male-centric moral systems often erase female presence. Rufus, shaped by suffering and rigid religious beliefs, insists, "'Satan,' he said. 'He has me in his power.'" (O'Connor, 1965), and later proclaims, "Nobody can save me but Jesus." (O'Connor, 1965), highlighting how fundamentalist ideas fill the moral vacuum left by parental neglect. The story stages the consequences of masculinity unchecked by emotional intelligence or relational understanding, ultimately showing that Sheppard's utilitarian

worldview fails both Rufus and his own son.

In *Indian Camp*, the Native American woman giving birth is unnamed and voiceless, her suffering rendered in stark, clinical terms. She "lay in the lower bunk, very big under a quilt. Her head was turned to one side," highlighting her physical vulnerability and emotional detachment from those around her (Hemingway, 1924). Her screams erupt as the men enter: "She screamed just as Nick and the two Indians followed his father and Uncle George into the shanty," yet no one addresses her directly; her voice is reduced to involuntary cries rather than meaningful dialogue (Hemingway, 1924). During the forced Caesarean, "Uncle George and three Indian men held the woman still," transforming her body into a passive site of masculine intervention (Hemingway, 1924). When she resists—"She bit Uncle George on the arm"—he responds with racist contempt: "Damn squaw bitch," a dehumanizing slur that the others laugh at (Hemingway, 1924). This surgical silence, viewed through a feminist lens, symbolizes the erasure of female agency, particularly that of Indigenous women. The woman's pain, muted and dismissed—"But her screams are not important"—and her unconsciousness after birth—"She did not know what had become of the baby or anything"—underscore her role as an object of labour and trauma, rather than a subject with autonomy (Hemingway, 1924). Hemingway's narrative thereby situates the Indigenous female body as a spectacle of suffering within a patriarchal, colonial framework.

Even *The Devil and Tom Walker* subtly critiques gender dynamics. Tom's wife is portrayed in harsh, caricatured terms: "a tall termagant, fierce of temper, loud of tongue, and strong of arm," whose voice "was often heard in wordy warfare with her husband" (Irving, 1824). Her greed rivals Tom's—"they even conspired to cheat each other"—but her punishment comes swiftly and grotesquely, as she disappears after attempting to strike her own bargain with the devil (Irving, 1824). Later, Tom discovers only "a heart

and liver tied up” in her check apron, while the narrator remarks, “a female scold is generally considered a match for the devil, yet in this instance she appears to have had the worst of it” (Irving, 1824). Her early demise clears the way for Tom’s own moral downfall, making space for his individual arc while rendering her merely a catalyst. A feminist reading might interpret her as a narrative scapegoat—punished for her assertiveness and erased to advance the male protagonist’s story. Her depiction as an overbearing wife whose fate is met with Tom’s indifferent relief—“Let us get hold of the property... and we will endeavour to do without the woman”—suggests that she functions more as a cautionary symbol than a character (Irving, 1824). In this sense, Irving’s tale reinforces patriarchal moral instruction by portraying female agency as dangerous and disposable, even as it unconsciously reveals the power that female presence holds in catalyzing narrative momentum.

Ultimately, these stories affirm literature’s capacity to confront mortality in all its dimensions—ethical, theological, psychological, and corporeal. Their differences reflect not just stylistic preference but deep shifts in how American culture understands life, death, and what it means to tell a story about either.

These layered perspectives—narratological, ethical, psychoanalytic, semiotic, deconstructive, and feminist—demonstrate the richness of American short fiction in grappling with death, morality, and the limits of understanding. Each story resists singular interpretation: Irving critiques capitalist hypocrisy under the guise of morality; O’Connor exposes the limits of secular liberalism; Poe questions the coherence of the self; Hemingway disassembles coming-of-age tropes into silent trauma.

By weaving together diverse theoretical frameworks, we gain a fuller picture of how these stories function—not just as narratives, but as ideological spaces where conflicting meanings, silences, and anxieties converge. These works continue to resonate precisely because they

destabilize our certainties, drawing us into the very questions they refuse to resolve.

COMPARATIVE DISCUSSION

A comparative exploration of the four selected American short stories—Irving’s *The Devil and Tom Walker*, O’Connor’s *The Lame Shall Enter First*, Poe’s *Ligeia*, and Hemingway’s *Indian Camp*—reveals key thematic and stylistic contrasts that enrich their individual narratives. All four stories grapple with the motif of death, yet their representations diverge in tone and purpose. In Irving and O’Connor, death serves a moral or spiritual function: Irving treats it as divine punishment for greed, while O’Connor frames it within Christian notions of redemption and grace. In contrast, Poe and Hemingway focus on the psychological and existential aspects. Poe’s story explores obsessive love and the boundary between life and death through Gothic horror, while Hemingway’s minimalist approach renders death as an unflinching, natural occurrence tied to human development.

Stylistically, the stories also differ markedly. Irving employs allegory and satire, while O’Connor uses Southern Gothic realism rich in religious symbolism. Poe’s dense, symbolic prose and use of the unreliable narrator exemplify the Gothic tradition’s introspective horror, whereas Hemingway’s sparse, journalistic style allows the trauma to resonate subtly but powerfully. These stylistic choices deeply influence the reader’s emotional and interpretive experience, showcasing the diverse ways American authors have used short fiction to explore universal human concerns.

These stories also mirror their historical moments—Irving reflects Enlightenment-era moralism, O’Connor Southern religiosity amid modern skepticism, Poe Victorian anxieties over mortality and the sublime, and Hemingway the emotional aftermath of war and modern disillusionment.

CONCLUSION

This research paper explores the portrayal of death across four iconic short stories, revealing the intricate interplay between cultural, historical, and personal contexts in shaping literary narratives. Washington Irving's *The Devil and Tom Walker* critiques greed and moral decay, offering death as divine retribution reflective of Puritanical values. Flannery O'Connor's *The Lame Shall Enter First* examines death through themes of redemption and grace, influenced by her Catholic worldview and the moral complexities of the American South. Edgar Allan Poe's *Ligeia* delves into the psychological and supernatural, presenting death as a mysterious force transcended by will and obsession. Meanwhile, Ernest Hemingway's *Indian Camp* portrays death starkly, contrasting the brutal realities of life with an exploration of innocence and initiation.

The analysis highlights the distinct literary techniques—ranging from allegory and gothic imagery to minimalism—that these authors employ to navigate universal themes. Together, these stories underscore the enduring relevance of death as a lens for understanding human nature, societal values, and existential questions. Future studies might explore how contemporary American short fiction continues to reframe death, especially in the context of digital life, climate anxiety, and shifting cultural values.

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