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Review Article

Aflatoxin: A major risk factor for hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is ranked among the most common leading causes of death from cancers worldwide. Majorly responsible for this is the potent mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxin. The risk factors are included to be hepatitis B and C viruses, HBV and HCV. Molecular mechanisms involved in a flatoxin and HBV/HCV-associated hepatocarcinogenesis include the complex interplay of genetic and epigenetic alterations that help in disrupting normal cellular processes with the action of cancerous traits. As aflatoxin triggers genetic mutations and epigenetic modifications through its activation and production of DNA adducts, while HBV and HCV infections lead to chronic hepatitis, oxidative damage, direct contact with host cellular machinery and favor the development of HCC. Mostly, aflatoxins are ingested through food products contaminated with this toxin, particularly grains and nuts, which are regularly consumed by populations using insanitary agriculture and storage of food. Once inside the body, aflatoxin B1 and similar metabolites have the ability to activate the cellular pathways associated with metabolic activation through cytochrome P450 enzymes that result in the creation of DNA adducts. These adducts lead to mutations within critical tumor suppressor genes like TP53 and activate oncogenes like RAS, causing tumorigenesis. Elucidating the complex molecular crosstalk between aflatoxin exposure and HBV/HCV infections is important for advancing targeted interventions to mitigate the global burden of HCC. This review consolidates the latest findings but sets a course for future research promisingly unravelling the complex interactions between aflatoxin exposure and HBV/HCV infections. It helps pave the way to good clinical outcomes and effective public health strategies as a means of combating hepatocellular carcinoma.

Keywords:

Hepatocellular Carcinoma, Aflatoxin, Genetic Mutations, Molecular Mechanisms.

1. Introduction

Hepatocellular carcinoma (HCC) ranks among the most prevalent and deadly cancers globally. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), along with exposure to aflatoxins, are primary etiological factors contributing to HCC [1, 2]. Aflatoxins, mainly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, contaminate crops such as maize, peanuts, and tree nuts, leading to human exposure through the consumption of contaminated food [3]. In the liver, aflatoxin B1 (AFB1) is metabolized by cytochrome P450 enzymes into a highly reactive form, AFB1-8,9-epoxide, which binds to DNA, forming mutagenic adducts [4]. This exposure initiates a biochemical cascade, resulting in

DNA adducts that specifically target the third base of codon 249 in the TP53 gene. This mutation causes a characteristic G to T transversion, which disrupts the tumor-suppressing function of p53, allowing cells to proliferate uncontrollably [4, 5]. Additionally, aflatoxins induce oxidative stress and activate inflammatory pathways, further damaging liver tissue and promoting carcinogenesis. HBV DNA integrates into the host genome, disrupting genes and regulatory elements crucial for cell growth and division, thus aiding oncogenesis [5, 6]. The HBV protein interacts with cellular pathways controlling apoptosis, the cell cycle, and DNA repair, and it influences epigenetic modifications such as DNA methylation and histone modification, leading to altered gene expression and tumor progression [6, 7]. HCV infection, on the other hand, causes chronic liver inflammation, leading to fibrosis and cirrhosis, significantly increasing the risk of HCC [8]. HCV proteins can promote cancer by altering signaling pathways related to apoptosis, cell prolif-

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eration, and immune response [9]. HCV also evades immune detection, resulting in continuous liver damage. Moreover, co-infection with HBV and HCV can synergistically exacerbate liver damage and accelerate the development of HCC.

Furthermore, dietary habits, genetic predispositions, and environmental factors also play significant roles in modulating the risk of HCC. Studies have shown that populations with diets high in aflatoxin-contaminated foods and low in essential nutrients such as antioxidants are at increased risk [10]. Genetic factors, including polymorphisms in genes involved in detoxification pathways, can influence individual susceptibility to aflatoxin-induced liver damage. Environmental factors, such as exposure to other hepatotoxins and lifestyle factors like alcohol consumption and smoking, can further compound the risk [11]. Understanding these multifaceted interactions is crucial for developing targeted prevention and intervention strategies. Public health efforts focusing on improving food safety, increasing vaccination coverage against HBV, and providing antiviral treatments for HBV and HCV infections are essential components in reducing the incidence of HCC. Additionally, advancing research into the molecular mechanisms underlying aflatoxin and viral hepatitis-associated hepatocarcinogenesis will pave the way for novel therapeutic approaches and improve clinical outcomes [12].

1.1. Epidemiological Research Insights

Numerous epidemiological studies have been conducted over the years to thoroughly explore the intricate link between aflatoxin exposure and liver cancer, particularly in regions that exhibit a high incidence of hepatocellular carcinoma (HCC), such as various countries in Asia and Africa [13]. In earlier studies, researchers faced significant limitations, which hindered their ability to accurately measure both aflatoxin exposure and the status of viral hepatitis in the populations they were examining [14]. These limitations often resulted in inconclusive findings and a lack of clear understanding of the relationship between aflatoxin and liver cancer risk. However, recent advances in the field of molecular dosimetry and improved methods for assessing viral infections have led to much clearer insights into this critical relationship [15]. These technological improvements have allowed scientists to better quantify aflatoxin exposure levels and assess the presence of viral hepatitis, resulting in more robust data. Multi-country studies conducted in regions such as China, the Philippines, Mozambique, and Swaziland have provided significant evidence of correlations between aflatoxin exposure and the incidence of liver cancer [5, 15]. These studies frequently highlight the existence of synergistic effects, particularly in conjunction with infections caused by Hepatitis B virus (HBV) and Hepatitis C virus (HCV) [16, 17]. This combination of factors has underscored the heightened risk for individuals exposed to aflatoxin in the presence of these viral infections, reinforcing the need for continued investigation and public health interventions in these affected regions [18].

The development of molecular biomarkers specifically designed to assess aflatoxin exposure has emerged as a significant and critical advancement in the field of toxicology and public health [19]. Among these biomarkers, aflatoxin-DNA

adducts and protein adducts have undergone thorough validation in multiple human populations across different geographic regions [20]. These innovative biomarkers serve as a direct and accurate measure of an individual's exposure to aflatoxin and the accompanying biological effects it may produce [3]. By facilitating this measurement, these biomarkers play a vital role in identifying individuals who are at a high risk for health issues related to aflatoxin exposure, thereby enhancing our ability to implement and evaluate preventive interventions effectively [21]. Numerous key studies have provided compelling evidence of the presence of aflatoxin-DNA adducts in biological samples such as urine and serum, with findings that correlate closely with the incidence of liver cancer [22]. This correlation underscores the importance of continued research in this area, as it can provide insights that are essential for designing targeted public health strategies aimed at reducing the prevalence of aflatoxin-related diseases.

1.2. Regional Demographics and Population Trends Analysis

This subsection will primarily concentrate on examining the geographical distributions and patterns associated with aflatoxin exposure and the corresponding incidence rates of liver cancer, specifically hepatocellular carcinoma (HCC). It will delve into several important population-based studies that have been meticulously conducted in regions known for their elevated rates of HCC, notably sub-Saharan Africa and Southeast Asia [23]. The discussion is set to include a comprehensive overview of the various methodologies employed to assess levels of aflatoxin exposure among these populations. This will encompass a detailed exploration of the prevalence rates of co-infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) within these demographics. Furthermore, the analysis will seek to establish and elucidate the statistical correlations that exist between aflatoxin exposure, the prevalence of these viral infections, and the incidence rates of HCC [24]. Significant insights derived from large-scale epidemiological studies will be presented, shedding light on the variations observed in exposure levels and the associated cancer risks experienced among different demographic groups [25]. Through this examination, the aim is to highlight the complex interplay between environmental factors, infectious disease co-morbidities, and cancer outcomes in diverse geographical settings [26].

2. Materials and Methods

To effectively understand and combat hepatocellular carcinoma (HCC) in relation to aflatoxin exposure and HBV/HCV infections, a comprehensive analysis of regional demographics and population trends is essential [27]. This study utilizes a range of innovative methods and materials to gather and analyze demographic and population data, ensuring a thorough understanding of the epidemiological landscape. Data sources include national and international databases such as the Global Burden of Disease (GBD) Database, World Health Organization reports, and Demographic and Health Surveys (DHS), along with local health registries and cancer registries that provide

detailed data on HCC incidence and mortality [28]. Hepatitis surveillance systems track HBV and HCV infection rates, treatment outcomes, and vaccination coverage [29]. Geographical Information Systems (GIS) are employed to visualize and analyze the spatial distribution of aflatoxin exposure, hepatitis virus prevalence, and HCC incidence, incorporating satellite imagery and remote sensing data to assess agricultural practices and environmental factors. The study focuses on targeted recruitment of high-risk regions, such as sub-Saharan Africa and Southeast Asia, ensuring diverse representation through stratified sampling based on age, gender, socio-economic status, and urban/rural residence as shown in Figure 1. Inclusion criteria encompass individuals from high-risk regions with documented aflatoxin exposure and HBV/HCV infection status, while exclusion criteria filter out individuals with other primary liver diseases or insufficient data on exposure and health outcomes [30]. Data collection techniques include structured interviews and standardized questionnaires to gather detailed demographic information, dietary habits, and medical history, along with biological sample collection of blood, urine, and, in some cases, liver biopsy samples for biomarker analysis [31]. Environmental sampling of food, soil, and water is conducted to assess sources of aflatoxin contamination [32]. Analytical methods encompass descriptive statistics to summarize demographic profiles and prevalence of exposure and disease, GIS mapping and hotspot analysis to identify regions with higher rates of exposure and disease [33], and statistical modeling such as multivariate regression and Bayesian hierarchical models to assess relationships between demographic factors, exposure, and HCC risk. Machine learning techniques like cluster analysis and predictive modeling are employed to identify subgroups with similar exposure patterns and predict HCC risk. Data quality and assurance measures include rigorous training for field staff, adherence to standard operating procedures (SOPs), utilization of electronic data capture (EDC) systems, and regular data audits. Ethical considerations ensure informed consent and participant confidentiality [34]. Advanced tools and technologies like remote sensing, satellite imagery, high-throughput sequencing, and wearable technology are integrated to monitor exposure and health parameters in real-time. The study also emphasizes risk communication through community engagement, health literacy materials, policy development for regulatory frameworks and public health guidelines, intervention programs for HBV vaccination and food safety, and enhanced surveillance systems for real-time monitoring [35]. By employing these innovative methods and materials, the analysis of regional demographics and population trends aims to provide a nuanced understanding of the factors contributing to HCC [36], ensuring that public health strategies and clinical interventions are effectively tailored to the specific needs and risks of different populations, ultimately enhancing outcomes and reducing the burden of liver cancer worldwide [37, 38].

3. Conclusion

In conclusion, analyzing regional demographics and population trends is crucial for understanding hepatocellular car-

S. No	Country/Region	Study Population	Methods	HCC Incidence Rate	Key Findings	References
1	China	Rural cohorts	Dietary surveys, urine biomarker (AFB1)	High (HBV prevalent)	Significant dose-response relationship between aflatoxin exposure and HCC risk.	[77, 78, 79]
2	Philippines	Urban and rural	Serum aflatoxin-albumin adducts	Moderate (20-30 per 100,000)	Synergistic effect of aflatoxin and HBV on HCC development.	[67, 79, 80]
3	Mozambique, Swaziland	Rural cohorts	Dietary surveys, urine biomarker (AFB1)	High (30-35 per 100,000)	High aflatoxin exposure linked with increased HCC incidence.	[80, 77, 81]
4	United States (Texas)	Migrant workers	Serum aflatoxin-albumin adducts	Low (5-10 per 100,000)	Lower aflatoxin exposure but notable in migrant populations with high HCV rates.	[82, 84, 27]

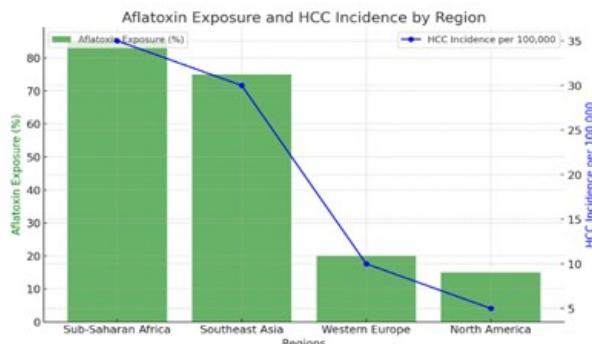


Figure 1: The bar chart above illustrates the percentage of population exposed to aflatoxins and the incidence of hepatocellular carcinoma (HCC) per 100,000 population across different regions. The data highlights the significant correlation between high aflatoxin exposure and increased HCC incidence, particularly in sub-Saharan Africa and Southeast Asia

cinoma (HCC) related to aflatoxin exposure and HBV/HCV infections. This study utilizes various data sources, such as health databases, cancer registries, and GIS tools, to explain the spatial prevalence of these risk factors. Targeted recruitment ensures diverse representation from high-risk areas, while structured interviews and sampling gather solid data on exposure and health effects. Advanced methods, including multivariate regression and machine learning, reveal significant relationships and predict HCC risk. Data integrity is maintained through rigorous quality assurance and ethical practices. Innovative technologies, like remote sensing and wearable devices, enhance monitoring of exposure and health metrics. This holistic approach helps develop targeted public health strategies and clinical interventions aimed at reducing liver cancer burdens in diverse populations, paving the way for better public health outcomes against HCC.

References

- D. Gouas, H. Shi, P. Hainaut, The aflatoxin-induced tp53 mutation at codon 249 (r249s): biomarker of exposure, early detection and target for therapy, *Cancer letters* 286 (1) (2009) 29–37.
- L. L. Bedard, T. E. Massey, Aflatoxin b1-induced dna damage and its repair, *Cancer letters* 241 (2) (2006) 174–183.
- C. P. Wild, Y. Y. Gong, Mycotoxins and human disease: a largely ignored global health issue, *Carcinogenesis* 31 (1) (2010) 71–82.
- J. Fung, C.-L. Lai, M.-F. Yuen, Hepatitis b and c virus-related carcinogenesis, *Clinical Microbiology and Infection* 15 (11) (2009) 964–970.
- M. Hifnawy, A. M. Mangoud, M. H. Eissa, E. N. Edin, Y. Mostafa, Y. Abouel-Magd, E. I. Sabee, I. Amin, A. Ismail, T. A. Morsy, et al., 8-the role of aflatoxin-contaminated food materials and hcv in developing hepatocellular carcinoma in sharkia governorate, egypt, *Journal-Egyptian Society Of Parasitology* 34 (1; SUPP) (2004) 479–488.
- M. Higgs, H. Lerat, J. Pawlotsky, Hepatitis c virus-induced activation of β -catenin promotes c-myc expression and a cascade of pro-carcinogenic events, *Oncogene* 32 (39) (2013) 4683–4693.
- Y.-J. Zhang, P. Rossner Jr, Y. Chen, M. Agrawal, Q. Wang, L. Wang, H. Ahsan, M.-W. Yu, P.-H. Lee, R. M. Santella, Aflatoxin b1 and polycyclic aromatic hydrocarbon adducts, p53 mutations and p16 methylation in liver tissue and plasma of hepatocellular carcinoma patients, *International journal of cancer* 119 (5) (2006) 985–991.
- H. Ming-Ju, H. Yih-Shou, C. Tzy-Yen, C. Hui-Ling, Hepatitis c virus e2 protein induce reactive oxygen species (ros)-related fibrogenesis in the hsc-t6 hepatic stellate cell line, *Journal of Cellular Biochemistry* 112 (1) (2011) 233–243.
- F.-s. Yeh, M. C. Yu, C.-C. Mo, S. Luo, M. J. Tong, B. E. Henderson, Hepatitis b virus, aflatoxins, and hepatocellular carcinoma in southern guangxi, china, *Cancer research* 49 (9) (1989) 2506–2509.
- I. Hsu, R. Metcalf, T. Sun, J. Welsh, N. Wang, C. Harris, Mutational hot spot in the p53 gene in human hepatocellular carcinomas, *Nature* 350 (6317) (1991) 427–428.
- F. Aguilar, C. Harris, T. Sun, M. Hollstein, P. Cerutti, Geographic variation of p53 mutational profile in nonmalignant human liver, *Science* 264 (5163) (1994) 1317–1319.
- H. Autrup, T. Seremet, J. Wakhsisi, A. Wasunna, Aflatoxin exposure measured by urinary excretion of aflatoxin b1-guanine adduct and hepatitis b virus infection in areas with different liver cancer incidence in kenya, *Cancer research* 47 (13) (1987) 3430–3433.
- M. C. Kew, Synergistic interaction between aflatoxin b1 and hepatitis b virus in hepatocarcinogenesis, *Liver international* 23 (6) (2003) 405–409.
- L. Ming, S. S. Thorgeirsson, M. H. Gail, P. Lu, C. C. Harris, N. Wang, Y. Shao, Z. Wu, G. Liu, X. Wang, et al., Dominant role of hepatitis b virus and cofactor role of aflatoxin in hepatocarcinogenesis in qidong, china, *Hepatology* 36 (5) (2002) 1214–1220.
- D. L. Palliyaguru, F. Wu, Global geographical overlap of aflatoxin and hepatitis c: controlling risk factors for liver cancer worldwide, *Food Additives & Contaminants: Part A* 30 (3) (2013) 534–540.
- C. Sun, D. Wu, L. Wang, C. Chen, S. You, R. Santella, Determinants of formation of aflatoxin-albumin adducts: a seven-township study in taiwan, *British journal of cancer* 87 (9) (2002) 966–970.
- S. Z. Iqbal, M. R. Asi, A. Ariño, N. Akram, M. Zuber, Aflatoxin contamination in different fractions of rice from pakistan and estimation of dietary intakes, *Mycotoxin research* 28 (2012) 175–180.
- W. Ohishi, S. Fujiwara, J. B. Cologne, G. Suzuki, M. Akahoshi, N. Nishi, I. Takahashi, K. Chayama, Risk factors for hepatocellular carcinoma in a japanese population: a nested case-control study, *Cancer Epidemiology Biomarkers & Prevention* 17 (4) (2008) 846–854.
- M. I. Shariff, I. J. Cox, A. I. Gomaa, S. A. Khan, W. Gedroyc, S. D. Taylor-Robinson, Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics, *Expert review of gastroenterology & hepatology* 3 (4) (2009) 353–367.
- J. H. Williams, T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly, D. Aggarwal, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, *The American journal of clinical nutrition* 80 (5) (2004) 1106–1122.
- T. Colin Campbell, J. Chen, C. Liu, J. Li, B. Parpia, Nonassociation of aflatoxin with primary liver cancer in a cross-sectional ecological survey in the people's republic of china, *Cancer Research* 50 (21) (1990) 6882–6893.
- C. Wild, Y.-Z. Jiang, S. Allen, L. Jansen, A. Hall, R. Montesano, Aflatoxin-albumin adducts in human sera from different regions of the world, *Carcinogenesis* 11 (12) (1990) 2271–2274.
- W.-X. Peng, J. Marchal, A. Van der Poel, Strategies to prevent and reduce mycotoxins for compound feed manufacturing, *Animal Feed Science and Technology* 237 (2018) 129–153.
- S. A. Gonzalez, J. F. Trotter, The rise of the opioid epidemic and hepatitis c-positive organs: A new era in liver transplantation, *Hepatology* 67 (4) (2018) 1600–1608.
- J. I. Tsui, E. C. Williams, P. K. Green, K. Berry, F. Su, G. N. Ioannou, Alcohol use and hepatitis c virus treatment outcomes among patients receiving direct antiviral agents, *Drug and alcohol dependence* 169 (2016) 101–109.
- Y. Liu, F. Wu, Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment, *Environmental health perspectives* 118 (6) (2010) 818–824.
- T. Chen, J. Liu, Y. Li, S. Wei, Burden of disease associated with dietary exposure to aflatoxins in china in 2020, *Nutrients* 14 (5) (2022) 1027.
- W. Cao, P. Yu, K. Yang, D. Cao, Aflatoxin b1: Metabolism, toxicology, and its involvement in oxidative stress and cancer development, *Toxicology Mechanisms and Methods* 32 (6) (2022) 395–419.
- L.-N. Qi, T. Bai, Z.-S. Chen, F.-X. Wu, Y.-Y. Chen, B. De Xiang, T. Peng, Z.-G. Han, L.-Q. Li, The p53 mutation spectrum in hepatocellular carcinoma

noma from guangxi, china: role of chronic hepatitis b virus infection and aflatoxin b1 exposure, *Liver international* 35 (3) (2015) 999–1009.

[30] S. Z. Iqbal, M. R. Asi, S. Jinap, Natural occurrence of aflatoxin b1 and aflatoxin m1 in halva and its ingredients, *Food Control* 34 (2) (2013) 404–407.

[31] S. Majeed, M. Iqbal, M. R. Asi, S. Z. Iqbal, Aflatoxins and ochratoxin a contamination in rice, corn and corn products from punjab, pakistan, *Journal of Cereal Science* 58 (3) (2013) 446–450.

[32] M. Alpert, M. S. R. Hutt, G. Wogan, C. Davidson, Association between aflatoxin content of food and hepatoma frequency in uganda, *Cancer* 28 (1) (1971) 253–260.

[33] J.-G. Chen, P. A. Egner, D. Ng, L. P. Jacobson, A. Muñoz, Y.-R. Zhu, G.-S. Qian, F. Wu, J.-M. Yuan, J. D. Groopman, et al., Reduced aflatoxin exposure presages decline in liver cancer mortality in an endemic region of china, *Cancer prevention research* 6 (10) (2013) 1038–1045.

[34] H. B. El-Serag, K. L. Rudolph, Hepatocellular carcinoma: epidemiology and molecular carcinogenesis, *Gastroenterology* 132 (7) (2007) 2557–2576.

[35] S. Roy, P. Banerjee, B. Ekser, K. Bayless, D. Zawieja, G. Alpini, S. S. Glaser, S. Chakraborty, Targeting lymphangiogenesis and lymph node metastasis in liver cancer, *The American Journal of Pathology* 191 (12) (2021) 2052–2063.

[36] M. C. Kew, Aflatoxins as a cause of hepatocellular carcinoma., *Journal of Gastrointestinal & Liver Diseases* 22 (3).

[37] P. Kumar, D. K. Mahato, M. Kamle, T. K. Mohanta, S. G. Kang, Aflatoxins: A global concern for food safety, human health and their management, *Frontiers in microbiology* 7 (2017) 2170.

[38] J.-S. Wang, J. D. Groopman, Dna damage by mycotoxins, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 424 (1–2) (1999) 167–181.

Research Article

Nanotherapeutic and stem cell therapeutic strategies in neurodegenerative diseases

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Abstract

In Neurodegenerative diseases the central or peripheral nerve systems gradually stop to perform proper functions. The treatments available are only help to relieve some physical or mental symptoms associated with these disorders. Due to its relatively extended duration, neurodegenerative disorders have become a major burden on families and society. Alzheimer's disease, Parkinson's disease (PD), and Huntington's disease (HD) are examples of neurodegenerative disorders that are either incurable or extremely difficult to treat. Therefore, new therapies are sought in which autologous stem cells are used. Stem cell therapy produces positive outcomes through a variety of pathways, including the direct replacement of lost or injured cells, the production of neurotrophic and growth factors and the stimulation of endogenous stem cells. However, low rates of stem cells differentiation and survival prevent them from being used in more therapeutic settings. Numerous intriguing nanomaterials for biomedical applications have been made possible by the quick development of nanotechnology. These materials are already widely used in the treatment of neurodegenerative diseases and appear to be able to make up for some of the shortcomings in stem cell therapy. Thus, a viable therapeutic method to treat neurodegenerative disorders is the combination of stem cell therapy and nanotherapeutic technologies.

Keywords:

Neurodegenerative diseases, Autologous Stem cells, Nanotherapeutic technologies.

1. Introduction

Neurodegenerative diseases involve the progressive neuronal loss and degeneration, development of disease-specific misfolded proteins, and finally the cognitive and sensory impairment. These comprises of multiple system atrophy (MSA), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and Alzheimer's disease [1]. The incidence of neurodegenerative diseases increases with age, and the incidence of sports-related traumatic brain injuries increases with time. Currently approved drugs for neurodegenerative diseases only temporarily reduce symptoms, but do not cure or slow the progression of the disease [2]. The lack of effective treatments causes a significant burden on society and large economic impacts. Regenerative cell therapy, sometimes referred to as stem cell therapy, has offered a great chance to investigate novel approaches that may be successful in treating neurodegenerative disorders over the past 20 years. This is because stem cells have the capacity to repair damaged nerve tissue by

replacing damaged or lost cells with differentiated cells, providing a favorable environment that supports regeneration, or protecting existing healthy neurons and glial cells from additional damage. The possibilities of stem cell therapy for neurodegenerative diseases were first explored in the 1980s when PD patients were treated with fetal mesencephalic tissue transplantation. Today, stem cell therapy offers promising strategies for the treatment of almost all neurodegenerative disorders. These strategies include regeneration of neural tissue, stabilization of neuronal networks, provision of neurotrophic support, and mitigation of neurodegeneration at the levels of different neuronal circuits [3]. For these debilitating neurological diseases, nanotechnology has shown great promise in overcoming the challenges presented by conventional therapies and promises revolutionary breakthroughs in their treatment. Quantum dots, polymer nanoparticles, carbon nanotubes, gold nanoparticles, liposomes, micelles, and fullerenes are some examples of the many nanoscale materials that have been developed and used in everything from advanced diagnostics to the delivery of neurotherapeutic agents and the evaluation of treatment efficacy. These nanomaterials can overcome such barriers, target a specific cell or signaling pathway, respond to body stimuli, transport genes

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and promote nerve regeneration. Treatment of neurodegenerative diseases may become possible using effective drug delivery methods such as these frameworks. Medicines encapsulated in nanomaterials have been found to treat diseases better than bulk materials used in traditional therapies. Thus, combining stem cell and nanotherapeutic approaches can enhance the treatment of neurodegenerative disorders and produce better clinical outcomes .

2. Stem cell therapy for parkinson disease

Parkinson's disease (PD) is the second most common neurological disease with a global prevalence of over 6 million for which there is yet no effective treatment available. It is predicted that during the next generation, the prevalence of Parkinson's disease would double. About 5% of those over 85 have Parkinson's disease (PD), as it is an age-related disorder. Alpha-synuclein (α -Syn) in the substantia nigra (SN) is the primary component of Lewy bodies (LBs), which are dopaminergic neurons that die in Parkinson's disease (PD). Because of the specific loss of dopaminergic neurons in the substantia nigra, Parkinson disease (PD) has long been regarded as one of the most promising disorders for cell replacement therapy. Over time, the idea of using stem cells to treat neurodegenerative disorders has changed, and it has recently advanced rapidly [4]. The most effective therapy for PD symptoms is dopamine replacement therapy (DRT). However, compensatory mechanisms for denervation alter and delay the relieving effect of DRT in a nonlinear way as the disease progresses. Additionally, surgical techniques such as deep brain stimulation and stereotactic ablations are used. However, in advanced Parkinson's disease patients, these methods only partially relieve the severe motor symptoms and DAergic desensitization that supports DRT. But neither surgical technique ends up being the main treatment. Early in the preclinical stage of research, scientists aimed to create a cell-based treatment using fetal ventral mesencephalon (fVM). Research on animals, which took place between 1977 and 1985, produced very positive outcomes using the 6-OHDA or MPTP lesion structure. either fetal tissue from humans or mice. The transplanted cells flourished in the damaged location, demonstrating the remarkable brain plasticity. Based on these findings, researchers have carried out a number of clinical experiments since 1987. The first trial involved a transplant carried out in Sweden, a nation that has an open stance regarding embryonic research. A wide range of open label trials and double-blind placebo-controlled trials conducted between 1987 and 2003 demonstrated that, generally, fVM transplantation was only slightly effective in relieving tremor in the majority of patients. But for a small percentage of patients, the procedure resulted failed to provide a quantifiable benefit. A wide variety of stem cells appears to be more promising and more probable to be created on a big scale when compared to fVM. More researchers are experimenting with stem cells in Parkinson's disease (PD) clinical trials, encouraged by evolving and improving technologies for cell derivation and differentiation. Benefits from stem cells include the ability to self-renew and their versatility in producing a wide range of tis-

sues. So, they are suitable substitutes for impaired nonrenewable neurons, particularly in the treatment of Parkinson's disease. In classical biology, stem cells are classified as either adult stem cells or ESCs based on their state of development. Additionally, stem cells can be divided into totipotent, pluripotent, and unipotent groups based on their level of developmental potential. The three main types of stem cells utilized to treat Parkinson's disease are neural stem cells (NSCs), mesenchymal stem and iPSCs. Neural stem cells (NSCs) are present in some regions of the human brain and persist throughout life. are present in two distinct brain regions and are present in both developing embryonic brain and the adult nervous system during human life. (Figure 1a) Under the normal conditions CNS Possess the capacity to be self-renew and differentiate into neurons, astrocytes, or oligodendroglia are in charge of restoring brain function .One potential alternative treatment for Parkinson's disease is the regeneration of dopaminergic neurons from stem cells. NSCs are a crucial component of the central nervous system (CNS). Adult endogenous NSCs are abundant in the lateral wall of the subventricular zone and the sub granular region of the dentate gyrus. These cells play a significant role in the central nervous system (CNS), helping to maintain the cell pool of neural tissues. A few essential characteristics of these cells are self-renewal, proliferation, and multipotency. NSCs may replace the lost DAergic neurons in Parkinson's disease (PD) by differentiating into neurons, astrocytes, or oligodendrocytes. Furthermore, because NSCs may move close to the site of damage to boost neuroblasts and aid in tissue healing, they can support endogenous repair mechanisms. NSCs can limit T cell proliferation and secrete cytokines, chemokines, and chemokine receptors, among other immunomodulatory actions [5] (Figure 1b).

Numerous preclinical investigations have demonstrated that many human NSC subtypes have the ability to move, survive, and multiply in particular brain regions following transplantation (Figure 1c). NSCs are involved in antiapoptotic, anti-inflammatory, and antioxidant brain repair processes.

The possibility that the transplanted human NSCs could be develop into functioning neurons and integrate into brain circuits is supported by immunohistochemical analyses. It has been shown through in vivo research that NSC transplantation is a successful PD treatment strategy. Compared to embryonic stem cells, MSCs have a number of benefits, such as a lesser immunological response, a decreased chance of tumor formation, and no ethical issues because they are adult stem cells with the ability to paracrine, immune control, and multidirectional differentiation. Since the identification of MSCs' immunomodulatory capabilities, further investigation has been conducted into the connection between inflammatory processes and MSCs. Evidence suggests that MSCs can use chemotaxis to migrate to the location of an inflammatory response. Once there, they can inhibit certain chemotaxis recruitment responses, which in turn lowers the inflammatory response in the damaged area and aids in tissue repair. Numerous autoimmune-related neurodegenerative and neuroinflammatory disorders, such as Parkinson's disease, Alzheimer's disease, multiple system atrophy, and amyotrophic lateral sclerosis, have been treated with

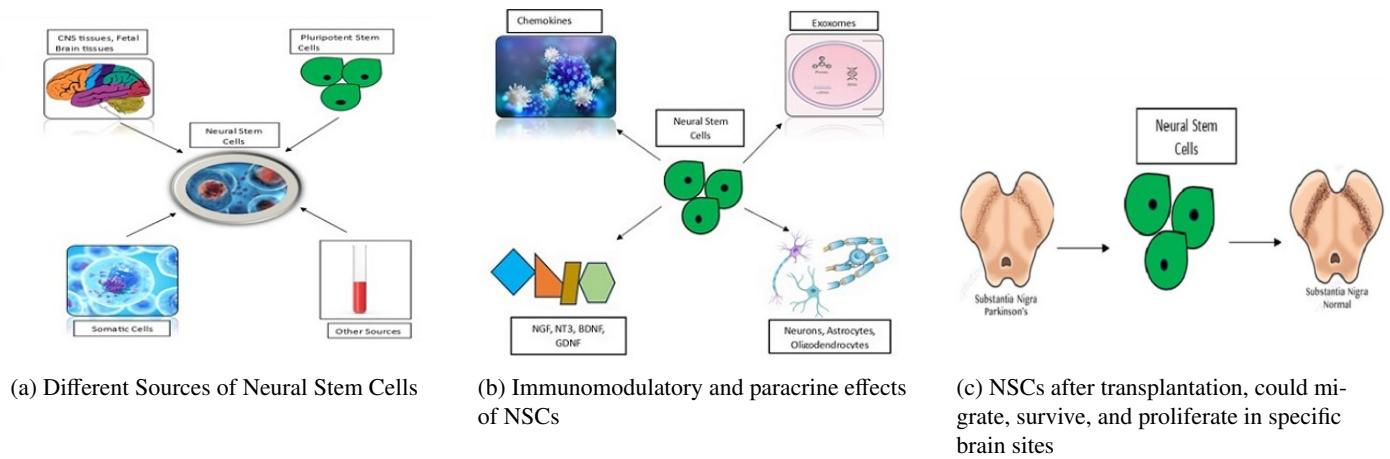


Figure 1

MSC-based cell treatments. The different multi-target disease modifying therapies of therapies of MSCs modulates α -Syn in PD is shown in Table 1.

3. Nanotherapeutic and stem cells based strategies for alzheimer's disease

Alzheimer Disease (AD) is the most common kind of dementia, affecting about 35 million individuals worldwide and exhibiting an upward trend. Unfortunately, no treatment strategies or preventative measures are now available for this progressive neurological condition. Since its first published description in 1907, tremendous progress has been made in understanding the molecular processes causing AD. Alzheimer's disease is characterized by the development of extracellular amyloid plaques, predominantly consisting of amyloid- β (A β), and intracellular neuro fibrillar tangles created by hyperphosphorylated tau proteins. These are both major clinical markers in the brains of AD patients. Clinical experiments aimed at treating Alzheimer's disease, including A β production and aggregation, have had minimal effectiveness. AD is one of roughly 40 amyloidosis, defined by the abnormal deposition of endogenous proteins as amyloid fibrils. It remains a complicated central nervous system condition with no known treatment. The protective blood-brain and blood-cerebrospinal fluid barriers complicate the treatment of Alzheimer disease, limiting the efficacy of several therapeutic treatments. However, the developing science of nanomedicine provides a potential approach to Alzheimer's disease diagnostics and therapy. Recent advances in nanomedicine have allowed for successful medication delivery to the brain, surpassing earlier hurdles. However, challenges remain in translating these advances into viable clinical applications for Alzheimer's disease therapy [6]. In the larger context of central nervous system (CNS) illnesses, NDs such as Alzheimer's disease, Parkinson's disease, epilepsy, and others contribute considerably to worldwide morbidity and mortality. According to recent forecasts, 416 million people are affected by Alzheimer's disease, emphasizing the need for improved treatments. Despite the well-known neuronal features of

Alzheimer's disease, existing therapies only give brief respite, and medications aimed at cognitive deficiencies encounter problems such as poor solubility, limited bioavailability, and hurdles along the drug delivery route. Nanotechnology-based platforms, such as polymeric NPs, dendrimers, and lipid-based NPs, provide potential alternatives for drug administration across the blood-brain barrier.

3.1. Nanotherapeutic Strategies

In recent years, nanotherapeutic techniques have emerged as viable options for diagnosing and treating Alzheimer's disease (AD). This article investigates several nanomaterials and their uses in Alzheimer's disease therapy, with an emphasis on medication transport, protein misfolding and aggregation, imaging, and diagnostic techniques. One of the most significant obstacles in AD therapy is the low permeability of substances via the BBB [7]. Nanomaterials act as efficient transporters for medications, genes, and cells, providing a solution to this problem. Functionalized targeting ligands, such as RGD peptides and transferrin, improve the targeting specificity of nanocarriers, enabling intelligent drug delivery systems. Furthermore, nanocarriers can provide regulated medication release by self-degradation or external stimulation, reducing drug dose while increasing effectiveness.

Here some organic nanoparticles used in Alzheimer's drug delivery: Liposomes the small vesicular structures comprised of lipid bilayers, play a significant role in Alzheimer's drug delivery. Their unique self-assembling and amphiphilic properties have led to FDA approval, establishing their effectiveness as drug carriers. A major advantage is their versatility, enabling the encapsulation of both hydrophobic and hydrophilic compounds within their lipid layers. Polyethylene glycol (PEG)-coated liposomes have shown impressive abilities in evading the reticuloendothelial system (RES). This evasion is essential for ensuring prolonged circulation in the bloodstream, thereby increasing the likelihood of drug transport across the blood-brain barrier (BBB). PEGylation enhances the stealthiness of liposomes, preventing undesired clearance by the immune system and facilitating targeted delivery to the brain [8]. Alzheimer's

drug delivery has seen significant advancements with the introduction of glutathione-PEGylated liposomes. These liposomes utilize the unique properties of glutathione to efficiently navigate through the cellular environment, resulting in enhanced cellular uptake across the blood-brain barrier (BBB). This innovative approach not only overcomes the challenges associated with selective permeability but also facilitates the targeted delivery of therapeutic agents. Liposomal drug delivery has already demonstrated successful applications in Alzheimer's treatment. Specifically, tailored liposomal formulations have been utilized to effectively deliver therapeutic agents such as coenzyme Q and nerve growth factor (NGF). For instance, coenzyme Q has been successfully delivered using chitosan-conjugated polylactide-polyglycolide (PLGA) nanoparticles. The conjugation with chitosan enhances the interaction of liposomes with biological systems, ensuring efficient drug transport. This highlights the potential of organic nanoparticles, particularly liposomes, as carriers for crucial Alzheimer's therapeutics[8]. In another significant application, polysorbate 80-coated polybutyl cyanoacrylate (PBCA) liposomes have been employed for the delivery of NGF. The surface modulation with polysorbate 80 improves the ability of liposomes to traverse biological barriers, underscoring the versatility and adaptability of liposomal formulations for targeted drug transport. Polymeric nanoparticles (NPs), such as PLGA and PAMAM dendrimers, have emerged as adaptable instruments for personalized drug administration in the setting of Alzheimer's disease. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles, when selectively functionalized with chitosan, demonstrate a novel strategy to Alzheimer's medication delivery. This alteration allows for efficient adsorption-mediated endocytosis across the blood-brain barrier (BBB). Chitosan, a natural polysaccharide, improves the interaction of PLGA NPs with cellular components, resulting in effective drug delivery. This technique demonstrates the versatility of polymeric nanoparticles in overcoming biological barriers for targeted medication delivery. Polyamidoamine (PAMAM) dendrimers with biocompatible hydroxyl groups offer a paradigm change in reducing invasiveness while increasing drug movement across the BBB. This breakthrough minimises damage to biological processes while efficiently conveying medicinal effects. While cationic amine group dendrimers have showed promise in drug delivery, worries about their possible toxicity have led researchers to investigate biocompatible alternatives. The search for safer dendrimeric structures demonstrates a dedication to improving medication carriers for Alzheimer's treatment. This emphasis on biocompatibility reflects continuous attempts to assure the safety and efficacy of polymeric nanoparticles in therapeutic settings. Carbon nanotubes (CNTs), known for their hydrophobic nature, are coated with chemical conjugates or hydrophilic biological molecules to improve their stability and functioning. Multi-walled carbon nanotubes functionalized with amine groups (MWCNTs-NH₃⁺) offer an appealing method for crossing the BBB via transcytosis. This method produces good results in both *in vitro* and *in vivo* experiments, establishing carbon nanotubes as a feasible inorganic carrier for Alzheimer's medication delivery. Gold nanoparticles (AuNPs), especially when coupled with wheat germ agglutinin horseradish perox-

idase (WGA-HRP), are effective at transporting medications over the BBB by intramuscular injection. This novel use of gold nanoparticles demonstrates their promise as efficient carriers for tailored medication delivery to particular brain areas. Inorganic nanoparticles, notably gold-based structures, provide alternate and promising approaches to circumventing BBB problems in Alzheimer's therapy[8].

The use of polymeric and inorganic nanoparticles represents a comprehensive approach to Alzheimer's medication delivery. These improvements, which range from modifying polymeric carriers for increased specificity to harnessing unique features of inorganic structures, highlight the changing landscape of nanotherapeutic techniques for tackling the intricacies of neurodegenerative illnesses. The blood-brain barrier (BBB) is a severe barrier to effective medication delivery to the brain, demanding novel nanotherapeutic approaches. Various ways have been taken to improve medication distribution across the BBB, resulting in a complex and dynamic environment in the search for successful Alzheimer's disease treatment. Lipophilic nanoparticles (NPs) serve an important role in improving medication delivery across the BBB. This is accomplished by endocytosis or lipophilic transcellular routes, which take advantage of the BBB's selective permeability. [18] The adsorptive characteristic of NPs towards blood capillaries provides long-term release, enhancing the chance of effective drug delivery. This technique emphasizes the need of designing nanocarriers to capitalize on lipophilic interactions for effective BBB penetration.

Liposomes, as flexible nanocarriers, may be efficiently functionalized and surface-modulated to improve medication delivery to the brain. Researchers use polyether, functional proteins, and cell-penetrating peptides (CPPs) to increase liposome interaction with the BBB. Polyethylene glycol (PEG) coating and glutathione-PEGylation appear as important strategies for improving cellular absorption and overcoming possible hurdles. [17] The capacity to change liposomal surfaces demonstrates the versatility of organic nanocarriers in crossing the BBB. Functionalized nanocarriers serve an important role in initiating receptor-mediated transcytosis and carrier protein-mediated transport of therapeutic candidates across the BBB. Cell-penetrating peptides (CPPs), such as SynB and Angiopeps, act as molecular facilitators in this process. These peptides allow small molecule medications to cross the BBB, bypassing its barriers. Recently, graphene quantum dots (GQDs) have been explored, which work as a carrier for the drug molecule and, due to their tiny size, may easily carry it into the brain. Specifically, ctGQDs were able to cross the BBB due to their tiny size and were helpful in lowering Alzheimer-like symptoms in animals. After successfully crossing the BBB, nanocarriers face the issue of targeted medication delivery inside the complex milieu of the brain. Various solutions have been used to overcome this challenge: Certain nanocarriers, such as phospholipid complexes, show a propensity for inflammatory areas in the brain. This focused method guarantees that drugs reach particular areas linked with Alzheimer's disease. The capacity of nanocarriers to selectively target inflammatory indicators demonstrates a sophisticated grasp of disease-specific microenvironments. Nanocarriers can be coated with neutral zwitterions to reduce the non-

targeted interactions and increase target specificity. This change adds a protective layer, reducing unwanted interactions and providing more regulated medication administration. The use of the zwitterionic coatings appears to be a potential approach for the optimizing nanocarrier behavior after the BBB crossing.

Modifying coronated nanocarriers further refines medication delivery to particular sites. Tailoring the surface characteristics of nanocarriers after protein corona creation enables precise targeting. This dynamic approach reflects continuing attempts to tailor nanotherapeutics to recognized interactions and limitations in the brain's complex milieu. Finally, nanotherapeutic treatments for Alzheimer's disease use a comprehensive strategy, using both organic and inorganic nanocarriers. From using liposomes and polymers to investigating the possibilities of carbon nanotubes and gold nanoparticles, each nanomaterial has various benefits in overcoming BBB barriers. Ongoing nanotherapeutics research not only addresses the obstacles of BBB penetration, but also seeks to improve post-BBB crossing behaviors, with the potential to revolutionize Alzheimer's treatment. Nanotherapeutic techniques dynamic and developing character makes them critical actors in the search for more effective and focused remedies to neurodegenerative disorders.

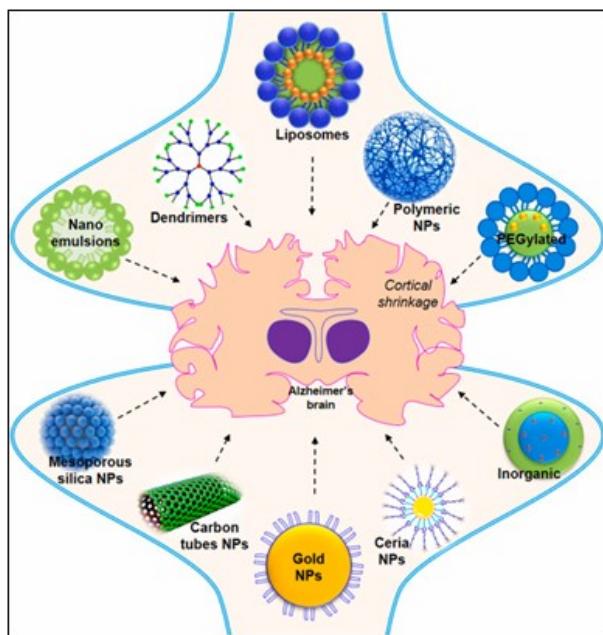


Figure 2: Various nanomaterials for treatment of Alzheimer's Disease

3.2. Stem cell based therapeutic strategies

Alzheimer's disease (AD) is a major global health concern, characterized by gradual cognitive loss and memory impairment. Traditional therapies have frequently focused on symptom management, but recent advancements in stem cell research provide intriguing pathways for new therapeutic techniques. This paper investigates the many techniques utilizing neural stem cells (NSCs), multipotent stem cells (MSCs), pluripotent stem

cells (PSCs), and the use of functional biomaterials in AD therapy [9].

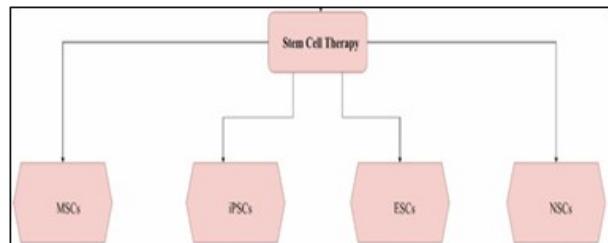


Figure 3: Different types of stem cells used for therapeutic purposes

3.2.1. Neural stem cells

The regenerative potential of NSCs has sparked interest in stem cell treatment for neurological illnesses such as Alzheimer's disease [10]. These self-renewing cells are critical for healing injured brain tissue. One strategy includes using endogenous NSCs found in the adult brain's hippocampus to trigger neurogenesis. Upregulation of growth hormones such as BDNF, IGF-1, NGF, and VEGF induces the patient's own NSCs to compensate for neurodegeneration. Despite its potential, but NSC transplantation has several hurdles, including ethical problems, safety concerns, and the need for standardization in clinic methods. Long-term culture may result in differentiation into glial cells, which reduces therapeutic efficacy. The variability of NSCs at various developmental stages complicates transplanting, emphasizing the necessity of purity, cell selection, and timing. To overcome these issues, continuing research is aimed at uncovering fundamental processes and devising innovative techniques. The use of bio scaffolds with NSC treatments shows potential. These scaffolds replicate the natural extracellular matrix (ECM) and provide an ideal environment for stem cell development, maturation, and transplantation. 2D and 3D hybrid inorganic nano scaffolds, such as those derived from MnO₂ nanosheets, have variable biodegradation potential, which improves the efficacy of stem cell treatments. This novel strategy overcomes barriers to successful clinical stem cell therapy, encouraging a fresh viewpoint on the use of NSCs in AD treatment [9]

3.2.2. Multipotent stem cells

MSCs obtained from diverse tissues provide an appealing option in Alzheimer's disease therapy. MSCs isolated from bone marrow, adipose tissue, and peripheral blood are versatile and have a wide variety of potential uses. Notably, fetal MSCs have better properties, such as increased telomerase activity and pluripotency factors, making them useful for bone tissue engineering and other therapeutic techniques. Clinical research to assess the safety and effectiveness of several MSC types, including umbilical cord blood-derived MSCs and adipose-derived stromal vascular fraction cells, are under underway. These experiments shed light on MSCs' potential for treating Alzheimer's disease. For example, in a rat model of Alzheimer's disease,

combining bone marrow-derived MSCs with particular particles had a strong protective effect. The continuous investigation of numerous MSC types and their uses highlights the need for more research to understand the processes, migratory features, and tumorigenic potential of these cells. Bioprinting methods, which use both natural and synthetic bioink materials, provide new opportunities for cell-based therapeutics in neurodegenerative illnesses. Bioink materials such as fibrin, collagen, gellan gum, poly(caprolactone), and poly(ethyleneglycole) serve as a foundation for novel microfluidic and bio plotting technologies. These advances improve cell survival and open up new possibilities for optimizing stem cell therapy for Alzheimer's disease.

3.2.3. Pluripotent stem cells and innovative biomaterials

The pluripotent stem cells, which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have a unique potential for treating Alzheimer's disease. ESCs, produced from the blastocyst's inner cell mass, have well-regulated pluripotency, allowing them to differentiate into a variety of cell types. The ethical, safety, and regulatory issues of using pluripotent stem cells to treat Alzheimer's disease must be carefully considered. Ongoing research and developments in stem cell technology, however, offer hope for creating novel medicines that address the underlying causes of Alzheimer's disease, possibly opening the door for later, more potent therapies. These cells can also be used to simulate the disease in culture dishes, which enables researchers to watch pathological processes and test possible treatments in a safe setting.

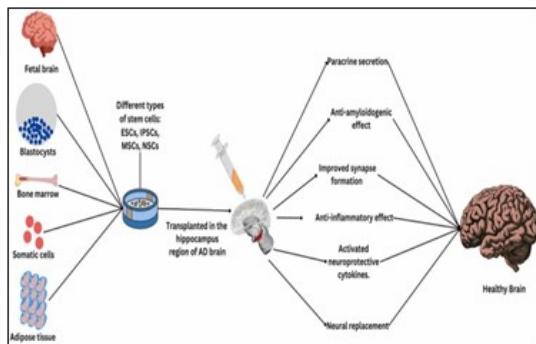


Figure 4: Injection of various types of stem cells being injected in the hippocampus area of brain and their benefits in clinical studies

Preclinical studies have shown that ESCs may successfully differentiate into cholinergic and GABAergic neuronal groups in AD models, leading to better memory impairment. However, the use of human ESCs has been linked to concerns about cancer development due to their strong proliferative capacity. To resolve ethical and safety concerns, researchers have resorted to iPSCs. iPSCs generated from mouse skin fibroblasts, treated with embryonic stem cell proteins, can develop into glial cells and decrease A_β plaques in transgenic AD mice models. Specific inducers such as Wnt, Fgf-8, BMP, Hedgehog, Notch, and retinoic acid are required for PSC development, including ESC and iPSC. The regulation of miRNAs and essential transcrip-

tion factors has been investigated for inducing somatic cell trans differentiation into neural stem cells and functioning neurons. The efficacy of stem cell treatments is determined not only by the features of the stem cells, but also by the techniques used to transfer them into host tissues. Innovative biomaterials have an important role in improving stem cell transplantation and encouraging therapeutic outcomes. Polymer-based materials have been created to aid in the nonenzymatic temperature-induced detachment of stem cells, assuring their gentle release for future uses. For example, homo polymeric coatings have been shown to allow the moderate thermally driven enzymatic-free selective release of undifferentiated iPSCs while leaving spontaneously developed cells attached to the surface. This technology purifies iPSCs quickly, inexpensively, and without the use of antibodies, which is critical for preserving maximum purity and homogeneity [10].

Bio scaffolds intended to replicate the natural extracellular matrix (ECM) provide a platform for increasing the efficacy of stem cell treatments. 2D and 3D hybrid inorganic nano scaffolds using materials like MnO₂ nanosheets provide variable biodegradation capability. These nano scaffolds promote stem cell differentiation, maturation, and transplantation by activating focal adhesion pathways and laminin binding. The adoption of such unique materials creates new opportunities for overcoming difficulties in clinical stem cell treatment, resulting in better outcomes [9].

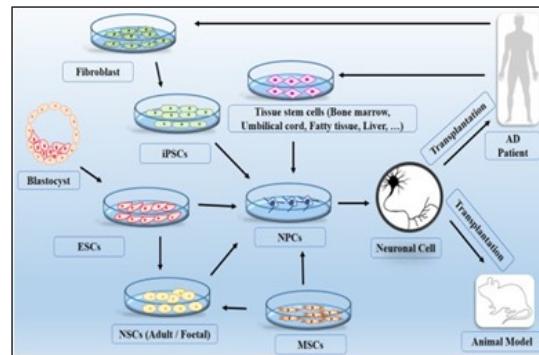


Figure 5: Various types of stem cells used in treatment of Alzheimer's Disease

3.2.4. Evaluating stem cell therapies in Clinical Trials

While stem cell-based therapy options for Alzheimer's disease are promising, moving these breakthroughs from preclinical investigations to clinical trials presents additional obstacles. Although most Alzheimer's disease cases are sporadic, transgenic animal models have mostly focused on familial AD. Despite failures, current clinical trials have evaluated the safety and effectiveness of several stem cell types in phase I trials. For example, a phase I experiment assessed the efficacy of human umbilical cord blood-derived MSCs delivered intracranially. Another experiment looked at the safety and efficacy of ASTOSTEM (autologous adipose tissue-derived mesenchymal stem cells) in 60 people and found that it improved their condition after delivery. Other trials, such as those using ischemia-tolerant human

bone marrow-derived MSCs, adipose-derived stromal vascular fraction cells, and umbilical cord blood-derived MSCs, are now in phase I/II

4. Collective use of nanotherapeutic and stem cell based strategies for treating alzheimer's disease and various other neurodegenerative disorders

Nanomaterials' dimensional features have a substantial impact on their physical, chemical, and biological properties. Nanomaterials are classified as zero dimensional (0D), one dimensional (1D), and two-dimensional (2D) structures. Nanoparticles and other zero dimensional nanomaterials are ideal for use as bioactive supplements, nanocarriers, or cell labels in stem cell treatment. On the other hand, one-dimensional (1D) or two dimensional (2D) nanomaterials, such as nanotubes, nanowires, graphene, and polymer membranes, have the potential to serve as 2D/3D nano scaffolds for stem cell differentiation. In the context to Alzheimer's disease, the combination of nanotherapeutic techniques with stem cell therapy offers a viable path to better treatment outcomes. While each strategy has advantages and limits in tackling the intricacies of Alzheimer's, their combination may provide complimentary benefits, increasing treatment success. The incorporation of stem cell therapy with nanotherapeutic methods has enormous promise for improving Alzheimer's disease treatment. When combined with stem cell therapy, zero-dimensional nanomaterials can act as useful supplements, transporters, and labelling agents. Simultaneously, the investigation of one-dimensional and two-dimensional nanomaterials, as well as their composites, as 2D/3D nano scaffolds shows potential for boosting stem cell development in the setting of Alzheimer's disease. In conclusion, the combination of nanotherapeutic techniques with stem cell treatment appears as a synergistic strategy with enormous potential for addressing the intricacies of Alzheimer's disease. This combination provides a holistic strategy that leverages nanoparticles' unique features and stem cells' regenerative capacity to improve treatment results.

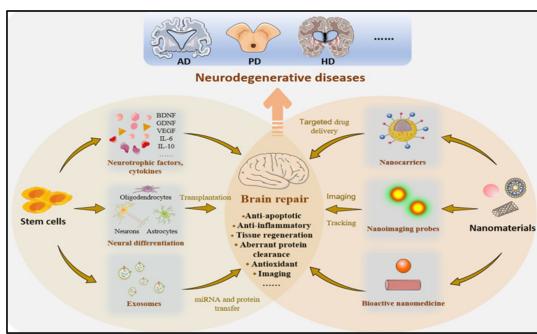


Figure 6: Schematic diagram showing use of stem cells therapy in collaboration with the nanotherapeutic strategies

5. Future perspectives

Despite advancements in clinical studies, stem cell treatments for Alzheimer's disease remain in their early phases. Several hurdles must be overcome in order to optimise these medicines for effective clinical use. These issues include the need for more studies to better understand the action mechanisms, migratory features, and tumorigenic potential of stem cells in the setting of AD. Unravelling the complexity is critical to developing focused and successful treatment methods. To ensure the safety and efficacy of stem cell treatments, processes for cell isolation, propagation, and transplantation must be standardized. To determine the safety profile of these medicines, their tumorigenic potential and physiological changes must be extensively evaluated. The inadequate and poor development of immature induced pluripotent stem cells (iPSCs) into somatic cells presents a difficulty [10]. Spontaneous differentiation into undesirable somatic cell types must be managed in order to improve the predictability and reliability of these treatments. The use of novel biomaterials, such as bio scaffolds and polymer-based materials, demands careful optimization. Balancing the requirement for effective stem cell distribution with regulated release while minimizing possible side effects is critical [11]. As clinical trials move to later stages, the design and quality of experimental research become more important. [24] Addressing experimental design concerns and providing rigorous procedures will be critical in assessing the protective benefits of stem cells in AD therapy. To summarize, the route towards using stem cells to treat Alzheimer's disease entails overcoming various difficulties. While continuing clinical trials give useful insights, collaboration among academics, physicians, and regulatory authorities is critical for navigating the complicated terrain of stem cell-based treatment options.

References

- [1] M. Wei, Z. Yang, S. Li, W. Le, Nanotherapeutic and stem cell therapeutic strategies in neurodegenerative diseases: a promising therapeutic approach, *International journal of nanomedicine* (2023) 611–626.
- [2] B. Martinez, P. V. Peplow, Biomaterial and tissue-engineering strategies for the treatment of brain neurodegeneration, *Neural Regeneration Research* 17 (10) (2022) 2108–2116.
- [3] F. Sivandzade, L. Cucullo, Regenerative stem cell therapy for neurodegenerative diseases: an overview, *International journal of molecular sciences* 22 (4) (2021) 2153.
- [4] F. Wang, Z. Sun, D. Peng, S. Gianchandani, W. Le, J. Boltze, S. Li, Cell-therapy for parkinson disease: A systematic review and meta-analysis, *Journal of translational medicine* 21 (1) (2023) 601.
- [5] A. Kamińska, K. Radoszkiewicz, P. Rybkowska, A. Wedzinska, A. Sarnowska, Interaction of neural stem cells (ncs) and mesenchymal stem cells (mcs) as a promising approach in brain study and nerve regeneration, *Cells* 11 (9) (2022) 1464.
- [6] N. Puranik, D. Yadav, M. Song, Advancements in the application of nanomedicine in alzheimer disease: a therapeutic perspective, *International Journal of Molecular Sciences* 24 (18) (2023) 14044.
- [7] T. Khan, R. Waseem, M. Shahid, J. Ansari, I. A. Ahanger, I. Hassan, A. Islam, Recent advancement in therapeutic strategies for alzheimer disease: Insights from clinical trials, *Ageing Research Reviews* 92 (2023) 102113.
- [8] H. Lv, Y. Wang, X. Yang, G. Ling, P. Zhang, Application of curcumin nanoformulations in alzheimer disease: Prevention, diagnosis and treatment, *Nutritional neuroscience* 26 (8) (2023) 727–742.

- [9] J. Gao, L. Li, Enhancement of neural regeneration as a therapeutic strategy for alzheimer disease, *Experimental and Therapeutic Medicine* 26 (3) (2023) 444.
- [10] Y.-H. Ban, D. Park, E.-K. Choi, T. M. Kim, S. S. Joo, Y.-B. Kim, Effectiveness of combinational treatments for alzheimer disease with human neural stem cells and microglial cells over-expressing functional genes, *International Journal of Molecular Sciences* 24 (11) (2023) 9561.
- [11] G. Gao, C. Li, Y. Ma, Z. Liang, Y. Li, X. Li, S. Fu, Y. Wang, X. Xia, J. C. Zheng, Neural stem cell-derived extracellular vesicles mitigate alzheimer disease-like phenotypes in a preclinical mouse model, *Signal Transduction and Targeted Therapy* 8 (1) (2023) 228.

Research Article

Effect of solvent on the extraction of constituents from the leaves of *Olea europaea* and their 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

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Abstract

Olive leaf extracts were prepared using the maceration method with a series of solvents of varying polarities, including n-hexane, dichloromethane, ethyl acetate, nitrobenzene, methyl ethyl ketone, acetone, methanol, and acetonitrile. Among the solvents, methanol yielded the highest extract content (26.8%), followed by nitrobenzene (18.4%), acetone (11.2%), ethyl acetate (10.4%), dichloromethane (9.2%), methyl ethyl ketone (6.8%), acetonitrile (3.6%), and n-hexane (0.96%). The antioxidant activity of these extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. At a concentration of 120 ppm, the percentage inhibition of DPPH radicals was highest for ascorbic acid (96.49%), followed by methanol (90.45%), ethyl acetate (85.58%), acetone (78.75%), and methyl ethyl ketone (66.28%). Lower inhibition was observed for acetonitrile (55.36%), nitrobenzene (39.77%), dichloromethane (38.01%) and n-hexane (28.07%). The antioxidant activity, evaluated using IC_{50} values (where lower values indicate higher antioxidant potential), identified ascorbic acid as the most potent standard ($IC_{50} = 7.03 \mu\text{g/mL}$). Among the samples, the n-hexane extract demonstrated the weakest antioxidant activity ($IC_{50} = 206.09 \mu\text{g/mL}$), while the methanol extract exhibited the strongest ($IC_{50} = 35.88 \mu\text{g/mL}$). Other extracts showed IC_{50} values of 157.77 $\mu\text{g/mL}$ for dichloromethane, 155.10 $\mu\text{g/mL}$ for nitrobenzene, 113.18 $\mu\text{g/mL}$ for acetonitrile, 80.98 $\mu\text{g/mL}$ for methyl ethyl ketone, 54.18 $\mu\text{g/mL}$ for acetone, and 40.29 $\mu\text{g/mL}$ for ethyl acetate. These results underscore the influence of solvent polarity on both extraction efficiency and antioxidant activity, with polar solvents generally yielding higher antioxidant potential.

Keywords:

Antioxidant activity, Bioactive compounds, DPPH, Polar solvents, extraction yield.

1. Introduction

The olive tree (*Olea europaea L.*), extensively cultivated in Mediterranean regions for centuries, is a key agriculture crop in Algeria and Tunisia, where around eight million hectares are devoted to its cultivation [1]. Olive industry generates a substantial volume of by-products, which can negatively impact the environment if not being managed and treated appropriately [2]. One of the primary by-products of the olive industry is olive leaves, which are generated during the pruning and harvesting of olive trees, and these leaves contain bioactive compounds that offer potential health benefits such as antioxidant activity, anti-HIV properties, anti-proliferative and apoptotic effects, protective effects against human leukemia, and lipid-lowering

activity [3, 4]. A synergistic effect has been reported among natural compounds, such as carotenoids, flavonoids, and other bioactive molecules with high antioxidant activity, in preventing damage to the body's cellular structures [5]. There is growing interest in natural antioxidants derived from plant materials. These are being explored as replacements for synthetic antioxidants [4]. Natural antioxidants are compounds that scavenge free radicals and can be derived from plants [6], microorganisms [7], or animals [8]. These antioxidants are often used to support the body's own antioxidants [9]. Inflammation and Oxidative stress (OS) are interconnected, so their interaction plays a significant role in the development of many of the world's leading diseases, such as neurodegenerative disorders [10], type 1 and type 2 diabetes, and cardiovascular diseases [11]. Free radicals have negative effects on public health, leading consumers to become increasingly mindful of their diet. As a result, they are adopting healthier consumption habits, including

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the intake of bioactive antioxidant compounds [12].

Olive tree is widely recognized as a species with exceptional antioxidant activity, found in its oil, fruit and leaves [13]. The medicinal and food industry applications of olive tree by-product extract are widely attributed to their rich antioxidant and phenolic compounds, preventing oxidative mutilation [14]. Polyphenols are significant class of bioactive secondary metabolites [15] that serve as anti-radical and antioxidants [16] which can also use in multiple purposes. Although much of the literature on olive polyphenols has primarily focused on olive oil as a key dietary source, olive leaves, which contain higher concentrations of phenolic compounds, are widely used in various fields, including pharmaceuticals, cosmetics, and the food industry [17]. Antioxidant compounds, such as phenolics, have shown significant effects in inhibiting the formation of Reactive Oxygen Species in organisms, thereby reducing the risk of chronic degenerative diseases like diabetics, cancer, atherosclerosis, and Alzheimer's [18, 19]. Various traditional and innovative methods are available for extracting phenolic compounds from plant materials [20, 21]. Soxhlet, maceration, and hydro distillation are regarded as conventional and reference techniques for extracting bioactive components [21], whereas ultrasound-assisted extraction takes less than one hour and yields 6 - 35 percent more than traditional methods, which require longer extraction times [22, 23].

This research aims to investigate the antioxidant activity and extraction yield of olive leaf extract by employing a range of solvents with varying polarities, from non-polar to high polar, in order to examine the influence of solvent polarity on the efficiency of extracting bioactive compounds. By utilizing the maceration method and analyzing the antioxidant potential through DPPH radical scavenging assay, the study seeks to elucidate the relationship between the physicochemical properties of solvents and their ability to extract polar bioactive molecules, such as phenolics and flavonoids, while also determining their antioxidant efficacy. Through this approach, the research highlights the critical role of solvent polarity in optimizing extraction processes and enhancing the antioxidant potential of natural extracts.

2. Materials and Methods

2.1. Chemicals and materials

Ethanol, n-hexane, dichloromethane, ethyl acetate, nitrobenzene, and methyl ethyl ketone were purchased from Thermo fisher Scientific, USA. Acetone, methanol, acetonitrile, and 1,1-diphenyl-2-picrylhydrazyl were taken from Sigma Aldrich, Germany. A Petri dish, beaker, volumetric flask, funnel, pipette, and graduated cylinder from Pyrex were used for measuring antioxidant activity and extraction leaves. Scott bottles (Duran), Whatman filter paper No.1 (Schleicher & Schuell), and electronic balance (Shimadzu) were used.

2.1.1. Preliminary treatment of sample

The leaves were washed with tap water to remove dust and extraneous particles and then allowed to dry in the shade. The

dried leaves were finely powdered using a blender at ambient temperature before being store in a polybag.

2.2. Extraction procedure

A simple maceration extraction process was acquired for these medicinal leaves. 25g of finely ground powder were macerated in 250mL of eight non-identical solvents on the basis of their ascending polarity order, in the context of their dipole moments (n-hexane 0.08, dichloromethane 1.14, ethyl acetate 1.88, nitrobenzene 3.9, methyl ethyl ketone 2.74, acetone 2.69, methanol 2.87, acetonitrile 3.44 D). The extraction was carried out under constant shaking for eight days, and the resulting extracts were filtered through Whatman filter paper to obtain an aqueous extract. The filtrates were then dried in a drying oven at 40°C, and the extraction yield was calculated using the following formula

$$Yield = \frac{W_d}{W_s} \times 100$$

W_d = weight of solvent-free extract

W_s = weight of raw material

2.3. Determination of antioxidant activity

Antioxidant activity of retrieved residues was evaluated by using 2,2-diphenyl-1-picrylhydrazyl [24]. The antioxidant activity of different extracts from olive leaves was measured using a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. 1mL portion of this solution was added to a flask containing 3mL of each extract from different solvents to prepare concentrations 20, 30, 40, 50, 60, and 80 µg/ml in ethanol. These solutions were kept in a dark place at room temperature for 30 minutes before measuring absorbance at 517nm using UV-Vis spectroscopy. Ascorbic acid of a similar concentration in ethanol was used as a reference standard. IC_{50} value of the extracted samples was determined using the logarithmic dose-response curve derived from the graph. A decrease in absorbance, attributed to antioxidant molecules reducing the strength of DPPH, indicated high radical scavenging activity. The percentage of free radical scavenging was calculated using the following equation [25].

$$\text{Percentage inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = DPPH and solvent

A_{sample} = DPPH and solution of extracted sample

3. Results and discussions

3.1. Solvent-dependent extraction yield

Figure 1 illustrates the extraction yields obtained using eight solvents arranged in order of increasing polarity (n-hexane to acetonitrile). The yields were influenced by the chemical nature of the solvents and their interactions with the constituents of olive leaves. Polar solvents like methanol (2.87D) and acetone dissolve polar compounds such as phenolics and flavonoids due

Table 1: Description and yield of crude extract

Extracted samples from solvents	Extraction Yield		Color
	(w/w) %	(w/w) g	
n-Hexane	0.96	0.240	Amber
Dichloromethane	9.20	2.300	Green
Ethyl Acetate	10.40	2.600	Seaweed
Nitrobenzene	18.40	4.600	Dark brown
Methyl ethyl ketone	6.80	1.700	Brown
Acetone	11.20	2.800	Greenish brown
Methanol	26.8	6.700	Blackish brown
Acetonitrile	3.60	0.900	Charcoal

to hydrogen bonding with hydroxyl and carbonyl groups. As a result, methanol provided the highest extraction (6.7g), reflecting its ability to dissolve a broad range of bioactive molecules, described in table 1. Polar solvents exhibit dipole-dipole interactions and hydrogen bonding with plant metabolites. High efficiency of methanol contrasts with acetonitrile (3.44D), which lacks hydrogen bonding capability, resulting in a lower yield (0.9 g). n-hexane (0.08D) had the lowest yield (0.24) due to its limited interaction with polar compounds while dichloromethane (1.14D) and ethyl acetate (1.88D) showed moderate yields (2.3 and 2.6 g), extracting both polar and non-polar compounds. Nitrobenzene (3.9D) produced an intermediate yield (4.6g) owing to aromatic interactions with lignins and flavonoids, despite its limited hydrogen bonding ability.

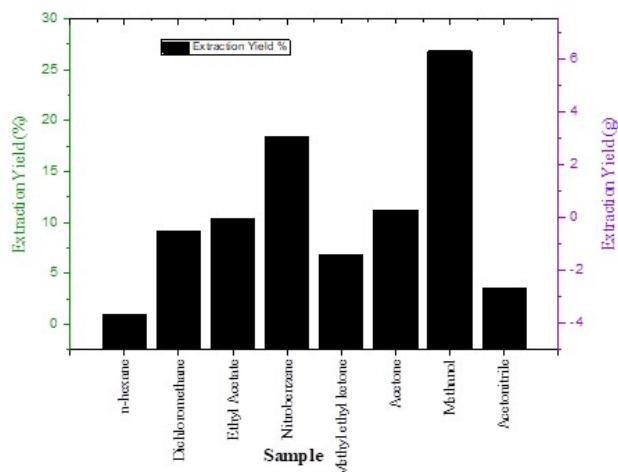


Figure 1: Yield of extraction from Olive leaves

3.2. Antioxidant activity of olive leaf extract by DPPH

The antioxidant activities of the samples were determined using DPPH assay after preparing dilutions in ethanol, ranging from 20 to 120 ppm. Ascorbic acid was used as a reference standard to compare the efficacy of the compounds. A constant volume of DPPH solution was mixed with each sample dilutions, allowing the reaction to occur. Since DPPH is a stable

radical, its interaction with antioxidant leads to color change from deep violet to light yellow, indicating the scavenging activities. The absorbance of the reaction mixtures was measured at 517 nm using UV-Vis spectrophotometer to determine the extent of free radical inhibitions. Additionally, the absorbance of the crude extracts, dissolved in solvents of varying polarities from n-hexane to acetonitrile, were recorded and presented in a Table 2 to analyze solvents effects on antioxidant potential.

Figure 2 shows minimum absorbance value indicates higher free radical scavenging activity, meaning that the antioxidant present in the sample effectively neutralize the free radicals, reducing the absorbance of the DPPH solution. The less the DPPH remains in its radical form, the higher the antioxidant activity of the samples. To quantify this activity, the percentage inhibitions were calculated and presented in the table.

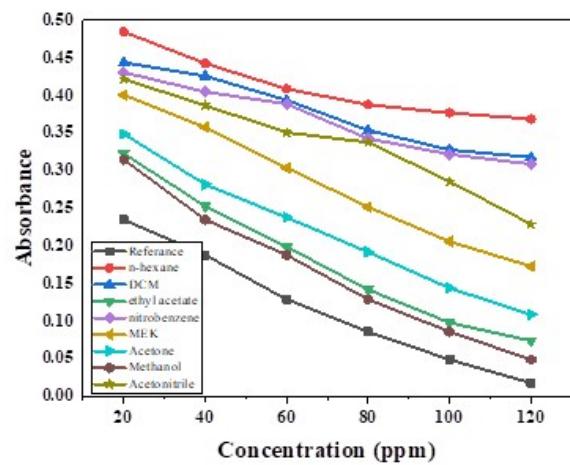


Figure 2: Absorbance for the determination of percentage inhibition

The percentage inhibition of DPPH radicals was determined using the standard equation having concentrations from 20 to 120 ppm as shown in Table 3, reflecting the antioxidant capacity of different solvent extracts based on their chemical properties. The observed trends align with the polarity principle in solvent extraction, where polar solvents like methanol, ethyl acetate and acetone demonstrate higher radical scavenging activity due to their ability to dissolve and extract polar antioxidant compounds, such as flavonoids and phenolics, through hydrogen bonding and dipole interactions. In contrast, non-polar solvents like n-hexane and nitrobenzene exhibited lower inhibition percentages due to Van der Waals interactions, depicted in Figure 3.

3.3. Oxidative stress-reducing properties of the extracts

The antioxidant activity of olive leaves extract was evaluated using solvents of varying polarities, as listed in Table 4: n-hexane, dichloromethane, ethyl acetate, nitrobenzene, methyl ethyl ketone, methanol, and acetonitrile. A standard antioxidant, ascorbic acid, was used for comparison with an IC_{50}

Table 2: Description and yield of crude extract

Conc. (ppm)	Ascorbic Acid	<i>n</i> -hexane	DCM	Ethyl acetate	Nitrobenzene	MEK	Acetone	Methanol	Acetonitrile
20	0.235	0.485	0.444	0.323	0.431	0.401	0.349	0.315	0.422
40	0.188	0.443	0.426	0.253	0.405	0.358	0.282	0.235	0.387
60	0.129	0.409	0.394	0.199	0.389	0.304	0.238	0.188	0.351
80	0.086	0.388	0.354	0.142	0.343	0.252	0.192	0.129	0.338
100	0.049	0.377	0.328	0.098	0.322	0.206	0.144	0.086	0.286
120	0.018	0.369	0.318	0.074	0.309	0.173	0.109	0.049	0.229

Table 3: Percentage inhibition of samples

Conc. (ppm)	Ascorbic Acid	<i>n</i> -hexane	DCM	Ethyl acetate	Nitrobenzene	MEK	Acetone	Methanol	Acetonitrile
20	54.19	54.46	13.45	37.04	15.98	21.83	31.97	38.60	17.74
40	63.35	13.65	16.96	50.68	21.05	30.21	45.03	54.19	24.56
60	74.85	20.27	23.20	61.21	24.17	40.74	53.61	63.35	31.58
80	83.24	24.37	30.99	72.32	33.14	50.88	62.57	74.85	34.11
100	90.45	26.51	36.06	80.90	37.23	59.84	71.93	83.24	44.25
120	96.49	28.07	38.01	85.58	39.77	66.28	78.75	90.45	55.36

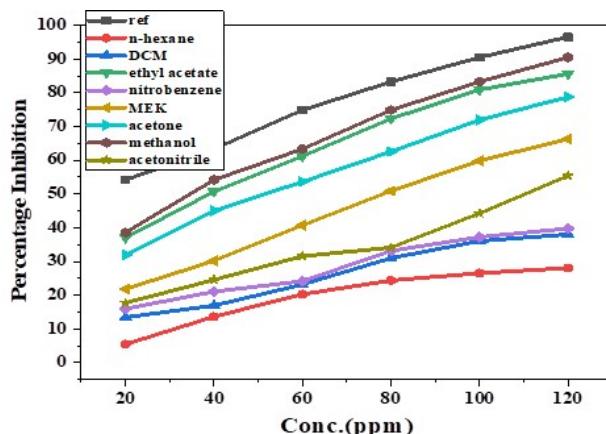


Figure 3: Percentage Inhibition

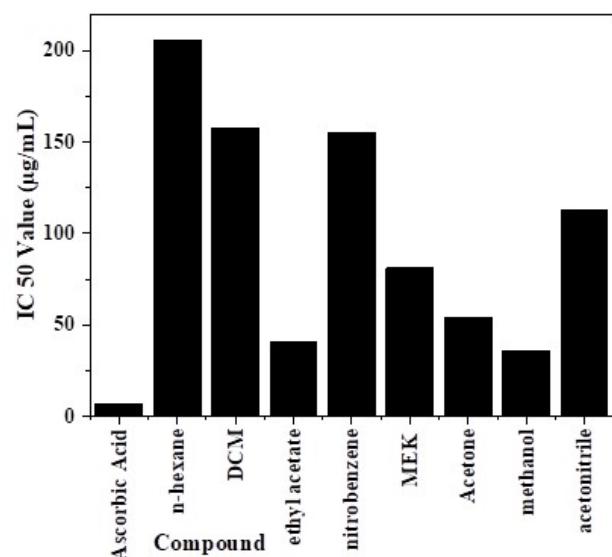
Table 4: IC_{50} Values of reference and samples

Compound extracted from solvents	IC_{50} Value ($\mu\text{g/mL}$)
Ascorbic Acid	7.03
n-Hexane	206.09
DCM	157.77
Ethyl acetate	40.29
Nitrobenzene	155.10
MEK	80.98
Acetone	54.18
Methanol	35.88
Acetonitrile	113.18

value of $7.03 \mu\text{g/mL}$. Among the solvents, shown in Figure 4, methanol ($IC_{50}=35.88 \mu\text{g/mL}$), acetone ($IC_{50}=54.18 \mu\text{g/mL}$) and ethyl acetate ($IC_{50}=40.29 \mu\text{g/mL}$) were exhibited strongest antioxidant activity, because of its ability to extract a wide range of polar bioactive compounds such as phenolic and flavonoids. Dichloromethane and nitrobenzene ($IC_{50}=157.77$ and $155.10 \mu\text{g/mL}$) were founded low antioxidant activity due to their moderately polar compounds like alkaloids and aromatic metabolites. Methyl ethyl ketone ($IC_{50}=80.98 \mu\text{g/mL}$) and acetonitrile ($IC_{50}=113.18 \mu\text{g/mL}$), despite high dipole moment of acetonitrile, showed lower efficiency compared to ascorbic acid. In contrast, non-polar solvents like n-hexane were yielded weak antioxidant activity ($IC_{50}=206.09 \mu\text{g/mL}$).

4. Conclusion

The results emphasize the importance of solvent selection in maximizing the yield and bioactivity of plant extracts. Polar solvents, due to their hydrogen bonding and dipole-dipole interaction capabilities, are more effective in extracting antioxidant-rich compounds. Methanol, most polar solvent used, achieved the highest extraction yield (26.8%) and the strongest antioxidant activity ($IC_{50} = 35.88 \mu\text{g/mL}$). In contrast, non-polar solvents such as n-hexane resulted both lower extraction yields and

Figure 4: IC_{50} values in $\mu\text{g/mL}$

weaker antioxidant activities. These findings confirm that polar solvents are more efficient in extracting phenolic compounds and flavonoids, which are key contributors to the antioxidant potential of olive leaves. The study underscores the potential of optimizing solvent systems to enhance the recovery of bioactive compounds for applications in pharmaceuticals, nutraceuticals, and functional foods.

References

- [1] I. Tarchoune, C. Sgherri, J. Eddouzi, A. Zinnai, M. F. Quartacci, M. Zarrouk, Olive leaf addition increases olive oil nutraceutical properties, *Molecules* 24 (3) (2019) 545.
- [2] M. Ghanem, W. A. Tawfik, E.-S. M. Mahdy, M. E. Abdelgawad, N. S. Abdel-Azim, M. M. El-Missiry, Chemical and biological evaluation of olive leaves as a waste by-product of olive oil industry, *Egyptian Pharmaceutical Journal* 18 (2) (2019) 172–177.
- [3] R. Abbattista, G. Ventura, C. D. Calvano, T. R. Cataldi, I. Losito, Bioactive compounds in waste by-products from olive oil production: Applications and structural characterization by mass spectrometry techniques, *Foods* 10 (6) (2021) 1236.
- [4] H. Benjeddou, C. B. Ahmed, B. B. Rouina, Influence of antioxidative enzymes, phytohormones and pigments in alternate bearing of three olive cultivars, *Scientia Horticulturae* 253 (2019) 17–23.
- [5] A. Muscolo, O. Mariateresa, T. Giulio, R. Mariateresa, Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases, *International journal of molecular sciences* 25 (6) (2024) 3264.
- [6] A. Altemimi, N. Lakhssassi, A. Baharlouei, D. G. Watson, D. A. Lightfoot, Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts, *Plants* 6 (4) (2017) 42.
- [7] A. Rani, K. C. Saini, F. Bast, S. Mehariya, S. K. Bhatia, R. Lavecchia, A. Zuorro, Microorganisms: a potential source of bioactive molecules for antioxidant applications, *Molecules* 26 (4) (2021) 1142.
- [8] J. Bechaux, P. Gatellier, J.-F. Le Page, Y. Drillet, V. Sante-Lhoutellier, A comprehensive review of bioactive peptides obtained from animal byproducts and their applications, *Food & function* 10 (10) (2019) 6244–6266.
- [9] I. Matic, A. Guidi, M. Kenzo, M. Mattei, A. Galgani, Investigation of medicinal plants traditionally used as dietary supplements: A review on *moringa oleifera*, *Journal of public health in Africa* 9 (3) (2018) 841.

[10] E. Scarian, C. Viola, F. Dragoni, R. Di Gerlando, B. Rizzo, L. Diamanti, S. Gagliardi, M. Bordoni, O. Pansarsa, New insights into oxidative stress and inflammatory response in neurodegenerative diseases, *International journal of molecular sciences* 25 (5) (2024) 2698.

[11] A. I. Balan, V. B. Halatiu, A. Scridon, Oxidative stress, inflammation, and mitochondrial dysfunction: a link between obesity and atrial fibrillation, *Antioxidants* 13 (1) (2024) 117.

[12] M. Ahmad, F. S. Punam, M. Amanullah, N. Sarwar, M. M. Haque, N. Yeasmin, S. Akther, Antioxidant activity and bioactive compounds of fruits available in bangladesh, *Health Dynamics* 1 (1) (2024) 22–29.

[13] M. Servili, M. Baldioli, R. Selvaggini, A. Macchioni, G. Montedoro, Phenolic compounds of olive fruit: one-and two-dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constitutive parts of fruit, *Journal of Agricultural and Food Chemistry* 47 (1) (1999) 12–18.

[14] P. G. Lins, S. M. P. Pugine, A. M. Scatolini, M. P. de Melo, In vitro antioxidant activity of olive leaf extract (*olea europaea l.*) and its protective effect on oxidative damage in human erythrocytes, *Heliyon* 4 (9).

[15] A.-N. Li, S. Li, Y.-J. Zhang, X.-R. Xu, Y.-M. Chen, H.-B. Li, Resources and biological activities of natural polyphenols, *Nutrients* 6 (12) (2014) 6020–6047.

[16] Y. Lang, N. Gao, Z. Zang, X. Meng, Y. Lin, S. Yang, Y. Yang, Z. Jin, B. Li, Classification and antioxidant assays of polyphenols: A review, *Journal of Future Foods* 4 (3) (2024) 193–204.

[17] M. De Bock, E. B. Thorstensen, J. G. Derraik, H. V. Henderson, P. L. Hofman, W. S. Cutfield, Human absorption and metabolism of oleuropein and hydroxytyrosol ingested as olive (*olea europaea l.*) leaf extract, *Molecular nutrition & food research* 57 (11) (2013) 2079–2085.

[18] N. Farnad, R. Heidari, B. Aslanipour, Phenolic composition and comparison of antioxidant activity of alcoholic extracts of peppermint (*mentha piperita*), *Journal of Food Measurement and Characterization* 8 (2) (2014) 113–121.

[19] G. Özkan, M. M. Özcan, Antioxidant activity of some medicinal plant extracts on oxidation of olive oil, *Journal of Food Measurement and Characterization* 11 (2) (2017) 812–817.

[20] J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jahurul, K. Ghafoor, N. A. Norulaini, A. K. Omar, Techniques for extraction of bioactive compounds from plant materials: A review, *Journal of food engineering* 117 (4) (2013) 426–436.

[21] S. Mollaei, F. Sedighi, B. Habibi, S. Hazrati, P. Asgharian, Extraction of essential oils of *ferulago angulata* with microwave-assisted hydrodistillation, *Industrial Crops and Products* 137 (2019) 43–51.

[22] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. mechanisms, techniques, combinations, protocols and applications. a review, *Ultrasonics sonochemistry* 34 (2017) 540–560.

[23] M. N. Safdar, T. Kausar, M. Nadeem, Comparison of ultrasound and maceration techniques for the extraction of polyphenols from the mango peel, *Journal of Food Processing and Preservation* 41 (4) (2017) e13028.

[24] M. Yamauchi, Y. Kitamura, H. Nagano, J. Kawatsu, H. Gotoh, Dpph measurements and structure-activity relationship studies on the antioxidant capacity of phenols, *Antioxidants* 13 (3) (2024) 309.

[25] L. L. Mensor, F. S. Menezes, G. G. Leitão, A. S. Reis, T. C. d. Santos, C. S. Coube, S. G. Leitão, Screening of brazilian plant extracts for antioxidant activity by the use of dpph free radical method, *Phytotherapy research* 15 (2) (2001) 127–130.

Research Article

Synthesis and antibacterial activity of synthesized derivative of sulphonamide drug of 1,3-thiazole

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Abstract

The amalgamation of two heterocyclic moieties i.e. 1,3-thiazole and 1,3,4-oxadiazole, were carried out in the designed molecules to impart them possible therapeutic properties. The new compounds have been synthesized by encompassing different bioactive moieties including 1,3-thiazole, sulphonyl, alkyl halid. The synthesized molecules have been subjected to evaluation of their antibacterial potential. Furthermore, enzyme inhibition potential results have been supported by computational docking in order to find the types of interactions with the active site of involved enzymes.

Keywords:

1,3-thiazole, alkyl halide, Sulphonamide, Antibiotic.

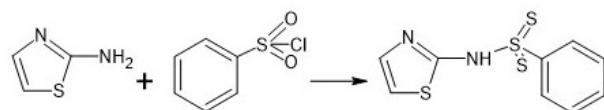
1. Introduction

The chemistry of heterocyclic compounds is a vast subject. Out of all the heterocyclic compound 1,3-thiazole is one of the important area of research because of the manifold pharmacological activities. It is used as an intermediate to manufacture synthetic drugs, fungicides, and dyes [1]. Its ring structure is also a useful element in medicinal chemistry. This structure has found applications in drug development for treatment of allergies, hypertension, inflammation, schizophrenia, and bacterial and HIV infections [2]. In the recent years, many thiazole derivatives have been synthesized and subjected to varied biological activities [3]. Naturally, thiazole is available in a large number of terrestrial and marine compounds with different pharmacological activities. Thiazole is also present in the vitamin B1 (Thiamine). It will be exciting to observe that these modifications can be utilized as potent therapeutic agents in future [4].

2. Materials and Methods

A simple method in aqueous media under dynamic pH control is adopted for synthesis of sulfonamides. Filtration after acidification is involved for isolation of products. All the

drugs were weighed accurately and dissolved completely by addition of distilled water by constant stirring using magnetic stirrer. The pH of the reaction contents was strictly monitored and maintained at 8-10 at regular intervals during the experimental reaction using Na_2CO_3 solution (1M). Then benzene sulphonyl chloride was accurately weighed and added carefully into the above solution. Reaction was carried in round bottom flask equipped with magnetic stirrer. Alkaline environment made the removal of hydrogen easier. During stirring benzene sulphonyl chloride initially floats on surface and the completion of reaction was examined by the change in pH value due to formation of HCl by the consumption of p-toluene sulphonyl chloride during the reaction. On completion of reaction pH was adjusted at 2-3 using HCl solution (2M). The precipitates formed were filtered through Whatman Filter Paper No. 42, washed several times with distilled water and recrystallized using methanol, and finally washed with water and acetone (9:1) and dried over anhydrous MgSO_4 . Products formation was confirmed through TLC (methanol:water:acetone in 60:20:20 ratio).

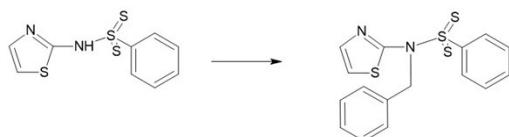


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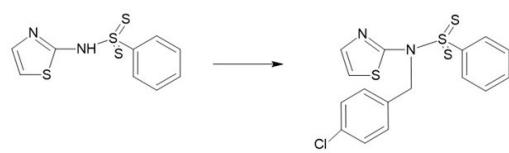
2.1. synthesis of *N*-benzyl-*N*-(1,3-thiazol-2-yl) benzenesulfonothioamide

The synthesized compound *N*-(1,3-thiazol-2-yl) benzene sulfonothioamide was dissolved in *N,N*-dimethyl formamide (DMF, 5-10 mL) in a 100 mL RB flask. Solid LiH (0.005g) was added and mixture was stirred for half an hour. Then different electrophiles, alkyl/aralkyl halides, were added in equimolar ratios and further stirred for 3-5 hours. The reaction was monitored by TLC using n-hexane and ethyl acetate solvent system (80:20). After completion of reaction, ice cold distilled water was added and the products were collected by filtration or solvent extraction.



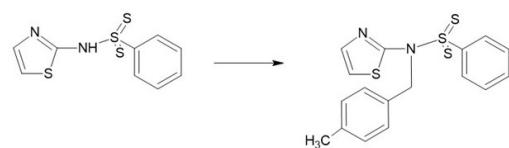
2.2. synthesis of *N*-[(4-chlorophenyl)methyl]-*N*-(1,3-thiazol-2-yl)benzenesulfonothioamide

The compound *N*-(1,3-thiazol-2-yl)benzenesulfonothioamide (17; 0.1g; 0.00047 mol) was dissolved in *N,N*-dimethyl formamide (DMF, 5-10 mL) in a 100 mL RB flask. Solid LiH (0.005g) was added and mixture was stirred for half an hour. Then different electrophiles, alkyl/aralkyl halides, were added in equimolar ratios and further stirred for 3-5 hours. The reaction was monitored by TLC using n-hexane and ethyl acetate solvent system (80:20). After completion of reaction, ice cold distilled water was added and the products were collected by filtration or solvent extraction



2.3. synthesis of *N*-[(4-methylphenyl)methyl]-*N*-(1,3-thiazol-2-yl)benzenesulfonothioamide

The compound *N*-(1,3-thiazol-2-yl)benzenesulfonothioamide (17; 0.1g; 0.00047 mol) was dissolved in *N,N*-dimethyl formamide (DMF, 5-10 mL) in a 100 mL RB flask. Solid LiH (0.005g) was added and mixture was stirred for half an hour. Then different electrophiles, alkyl/aralkyl halides, were added in equimolar ratios and further stirred for 3-5 hours. The reaction was monitored by TLC using n-hexane and ethyl acetate solvent system (80:20). After completion of reaction, ice cold distilled water was added and the products were collected by filtration or solvent extraction.



2.4. Antibacterial Assay

Disc diffusion method was used to find out the antibacterial activity of the synthesized compounds. 100 μ L suspensions of tested microorganisms was spread on PDA medium for 106 spores/mL of fungi and on NA medium for 107 colony-forming units/mL of bacteria cells. The filter discs of 6 mm diameter were saturated with compound solution and placed on the agar plates inoculated with the tested microorganisms. Filter discs without samples were employed as negative control. Fluconazole (30 μ g/disk) and Streptomycin (30 μ g/disk) were applied as positive reference for bacterial strains and fungal strains, respectively. Plates were placed 4° C for 2 hours and then incubated at 37° C for 18 hours for bacterial strains. Antimicrobial activity was justified after comparison of diameter of growth inhibition zone measured in mm for organisms and the controls [5, 6].

3. Results

3.1. *N*-benzyl-*N*-(1,3-thiazol-2-yl)benzenesulfonothioamide

Light brown solid; yield: 89%; m.p.: 236-237°C; Molecular Formula: $C_{13}H_{12}N_4OS_2$; Mol. Mass: 304 $gmol^{-1}$. IR (KBr, cm^{-1}) v: 3350 (N-H), 3052 (C-H of aromatic ring), 2923 (-CH₂- stretching), 1576 (C=C stretching of aromatic ring), 1518(C=N). EI-MS: m/z 304 [M]⁺, 233 [$C_{17}H_{15}N$]⁺, 213 [$C_6H_5N_4OS_2$]⁺, 170 [$C_7H_8NO_2S$]⁺, 155 [$C_7H_7O_2S$]⁺, 127 [$C_{10}H_7$]⁺, 113 [$C_4H_5N_2S$]⁺, 91 [C_7H_7]⁺

3.2. *N*-[(4-chlorophenyl)methyl]-*N*-(1,3-thiazol-2-yl)benzenesulfonothioamide

Dull white solid; yield: 87%; m.p.: 261-262°C; Mol. Formula: $C_{13}H_9ClN_4O_2S_2$; Mol. Mass: 352 $gmol^{-1}$. IR (KBr, cm^{-1}) v: 3345 (N-H), 3175 (C-H stretching of aromatic ring), 2923 (-CH₂- stretching), 1672 (C=C stretching of aromatic ring), 1590 (C=N), 743 (-C-H), 580 (C-Cl stretching). EIMS: m/z 352 [M]⁺, 253 [$C_{10}H_6ClN_2O_2S$]⁺, 226 [$C_9H_6ClN_2OS$]⁺, 193 [$C_8H_6N_3OS$]⁺, 179 [$C_8H_4ClN_2OS$]⁺, 139 [$C_5H_3N_2OS$]⁺, 125 [C_7H_5Cl]⁺, 114 [$C_3H_2N_2OS$]⁺, 75 [C_6H_3]⁺.

3.2.1. *N*-[(4-methylphenyl)methyl]-*N*-(1,3-thiazol-2-yl)benzenesulfonothioamide

Light-brown solid; yield: 79%; m.p.: 227-228 oC; Mol. Formula: $C_{15}H_{14}N_4O_2S_2$; Mol. Mass: 346 g/mol; IR (KBr, cm^{-1}) v: 3358 (N-H stretching), 2977 (C-H stretching of aromatic ring), 1675 (C=N stretching), 1645 (C=O stretching), 1576 (C=C stretching of aromatic ring), 1169 (C-O-C stretching). EI-MS: m/z 346 [M]⁺, 233 [$C_{11}H_9N_2O_2S$]⁺, 206 [$C_{10}H_9N_2OS$]⁺, 192 [$C_8H_5N_3OS$]⁺, 159 [$C_9H_7N_2O$]⁺, 141 [$C_5H_5N_2OS$]⁺, 119 [C_8H_7O]⁺, 104 [C_7H_4O]⁺, 91 [C_7H_7]⁺, 65 [C_5H_5]⁺.

Table 1: % Biofilm inhibition against *E. coli* and *S. aureus* of synthesized compounds a-c

Compound	% Inhibition	
	<i>E. coli</i>	<i>S. aureus</i>
a	31.278	18.392
b	9.692	40.503
c	30.250	24.673

4. Discussion

A series of three sulfonamides were synthesized in aqueous basic media by simple reaction of six amino group containing drugs; two amino acids and two amino acid analogs with paratoluene sulphonyl chloride with continuous stirring and details of reaction conditions are explained in experimental section. The compounds were obtained in excellent yield (above 80%). Elemental analysis was performed for the conformation of all the compounds and measurement of absorption maximum provided the justification. The synthesized compounds were characterized by FT-IR; the characteristics band at 3263-3371 cm^{-1} of N-H amide stretching and 1174-1127 cm^{-1} for (-N- S=O) and 1072-1010 cm^{-1} (S=O) for all compounds reveals the formation of sulfonamides. $[M + 2]^+$ peaks obtained by ESI-MS represented the isolation of sulfonyl group in all synthesized compounds. The structures of all the compounds were also confirmed by ^1H NMR and ^{13}C NMR by dissolving in MeOD. ^1H NMR spectra of compounds a,b and c showed a signal at δ 7.03-7.61, while a signal at δ 16.11 and 11.81?ppm for 11b and 11a corresponds to NH group of sulfonamide.

A broad singlet due to -NH group was also obtained for compounds 3a, 3b, 5b, and 13a at δ 8.38, 9.68, 8.01, and 9.51 ppm, respectively. The characteristics C-SO-NH signals at δ 131-139 ppm of all the compounds were shown by ^{13}C NMR which identified structures correctly. Synthesized compounds were also screened for their antibacterial activities against gram negative bacterial *E. coli* and gram positive *S. aureus* and *B. subtilis* by following the guidelines of CLSI [7, 8] using ciprofloxacin as reference antibacterial agent. Among the bacterial strains, the compounds 3a and 3b have excellent antibacterial activities against *S. aureus* with zone of inhibition comparable with control drug. Compounds c, b, a, and a showed moderate

activities while remaining compounds have no activity against the prescribed bacterial strain. Compounds a and b exhibited excellent activities against *E. coli* almost the same zone of inhibition as by reference ciprofloxacin (MIC 7.81), while 3a and 7a showed no activity.

5. Conclusion

In conclusion, three novel sulfonamides were synthesized; the reactions conditions are easy and excellent yields of compounds were obtained and progress of reaction was monitored by TLC and their structures were confirmed by spectral and elemental analysis. All the synthesized compounds were evaluated for their antibacterial activities and the results of their bioassay indicated that the sulfonamides attached to amino acid (histidine) and antifibrinolytic (tranexamic acid) showed antibacterial activities comparable to ciprofloxacin although these two agents alone have no antibacterial activity. The results confirmed that the compounds which are inactive against bacterial strains showed antibacterial activities after formation of sulfonamides.

References

- [1] A. Hantzsch, J. Weber, Ueber verbindungen des thiazols (pyridins der thiophenreihe), Berichte der deutschen chemischen Gesellschaft 20 (2) (1887) 3118–3132.
- [2] M. Bakavoli, H. Beyzaie, M. Rahimizadeh, H. Eshghi, R. Takjoo, Regioselective synthesis of new 2-(e)-cyano (thiazolidin-2-ylidene) thiazoles, Molecules 14 (12) (2009) 4849–4857.
- [3] R. Breslow, On the mechanism of thiamine action. iv. 1 evidence from studies on model systems, Journal of the American Chemical Society 80 (14) (1958) 3719–3726.
- [4] R. J. Fosbinder, L. Walter, Sulfanilamido derivatives of heterocyclic amines, Journal of the American Chemical Society 61 (8) (1939) 2032–2033.
- [5] S. Hazra, A review on synthetic strategies towards synthesis of thiazoles.
- [6] R. Lakhani, R. L. Singh, Synthesis and evaluation of 2-imino-3-(4-arylthiazol-2-yl)-4-thiazolidinones and their 5-arylidene derivatives as potential fungicides, Journal of agricultural and food chemistry 39 (3) (1991) 580–583.
- [7] T. Moulard, J. F. Lagorce, J. C. Thomes, C. Raby, Biological evaluation of compounds with-ncs-group or derived from thiazole and imidazole. activity on prostaglandin synthetase complex, Journal of pharmacy and pharmacology 45 (8) (1993) 731–735.
- [8] R. GUPTA, N. K. FULORIA, S. FULORIA, Synthesis and antimicrobial profile of some newer 2-amino-thiazole derivatives., Turkish Journal of Pharmaceutical Sciences 10 (3).

Research Article

Analysis of presentation attacks detection in signature biometrics

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Abstract

Authentication of biometric methods using various applications such as face or fingerprint recognition have received a lot of interest recently. However, at the same time these biometric systems have been facing different types of attacks. The current work carries out an analysis of different Presentation Attack (PA) scenarios for on-line handwritten signature verification. The present work introduces a short summary of methods for Presentation Attack Detection (PAD) in signature biometrics in order to describe the different levels of PAs existing in on-line signature verification regarding the amount of information available to the attacker, as well as the training, effort and ability to perform the forgeries. This work is an effort towards security evaluation of biometric systems, where attacks are rated depending on expertise of the attacker, as well as the information available and used from the target being attacked.

Keywords:

Presentation Attack Detection (PAD), On-line signature verification, Forgery.

1. Introduction

Applications relying on biometric user authentication have witnessed significant adoption across diverse sectors, including finance, healthcare, education, e-government, insurance, and security [1]. The surge in their popularity can be attributed to two major factors. Firstly, the advancement of sensor technology [2], leading to reduced costs of general-purpose devices like smartphones and tablets, which has increased societal acceptance. Secondly, the evolution of biometric recognition technologies in general [3–5]. However, it's crucial to acknowledge that these biometric-based authentication systems must be resilient against various potential attacks [6]. Our study primarily centers on exploring Presentation Attack (PA) scenarios concerning online handwritten signature biometric authentication systems. These systems have garnered considerable attention due to enhanced signature acquisition setups (including device interoperability [7]) and diverse writing inputs (e.g., finger [8]). In the context of signature verification, two types of impostors can be identified: (1) random (zero-effort or accidental) impostors, where the attacker possesses no information about the targeted user and presents their own signature as the user's, and

(2) skilled impostors, who have some level of information about the targeted user (e.g., an image of the signature) and attempt to forge their signature to deceive the system.

Recently, Galbally et al. [9] discussed different approaches for reporting accuracy in handwritten signature verification, incorporating insights gained from evaluating vulnerabilities in Presentation Attacks (PAs). They considered skilled impostors as a particular case of biometric PAs, akin to mimicry, a behavioral biometric characteristic. Notably, the distinction between physical PAs and mimicry lies in the fact that traditional PAs involve the use of physical artifacts like fake masks and gummy fingers (which can sometimes be detected at the sensor level), whereas mimicry involves the exact interaction observed in a normal access attempt. To align with the biometric Presentation Attack Detection (PAD) field, Galbally et al. [9] modified the nomenclature for impostor scenarios in signature verification, referring to the classical random impostor scenario as Bona Fide (BF) scenario and the skilled impostor scenario as the PA scenario.

Another approach to enhance security against attacks in signature biometrics, apart from employing a PAD module, is template protection [10]. Conventional on-line signature verification systems use highly sensitive biometric data such as the X and Y spatial coordinates for matching, storing this information as user templates without any form of protection. A

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compromised template in such a system could facilitate an attacker in generating high-quality forgeries of the original signature, as it provides the X and Y coordinates over time. A different approach [11] for signature template generation was proposed, omitting information related to X, Y coordinates and their derivatives, resulting in a more robust system against attacks, with comparable error rates to traditional systems that store sensitive information.

Subsequent studies [12] delved into PAD methods at the feature level for on-line signature verification. Yusof et. al. [13] introduced a new scheme that added a Skilled Forgeries Detector module to the original verification system. This module focused on detecting skilled forgeries based on four parameters of the Sigma LogNormal writing generation model and a linear classifier. The approach yielded promising results for both skilled (PA) and random (BF) scenarios. On the other hand, Reillo et al. [14] proposed PAD methods based on global features like the total number of strokes and signing time of signatures. They built a new database with 11 levels of PAs, and their proposed PAD significantly reduced the Equal Error Rate (EER).

Examining Different Levels of Presentation Attacks in Signature Biometrics: This section aims to explore various levels of skilled forgeries (PA impostors) found in the literature, considering the information available to the attacker, their training, effort, and forgery capabilities. Additionally, we consider random forgeries (zero-effort impostors), although they belong to the BF scenario, to encompass the entire range of possible impostors in handwritten signature verification.

Previous studies [15, 16] have applied the concept of Biometric Menagerie to categorize users of the biometric system based on animal classifications. The concept has been extended in recent research [17], considering various biometric modalities, including 2D and 3D faces, fingerprints, iris, speech, and keystroke dynamics. In on-line signature verification [16], the Biometric Menagerie concept was employed to classify users and quantify the difficulty of forging their signatures using personal and relative entropy measures.

Further research demonstrated [18] that some users are significantly better forgers (wolves) than others, and forgers can be trained to become a greater threat. Certain users are easy targets for forgers (sheep), and most individuals are relatively poor at judging handwriting authenticity. A new metric for impostor classification was proposed, distinguishing between naive, trained, and generative impostors.

Additional studies [19] developed software tools to generate forgeries of different quality levels (PA impostors). Three levels of PAs were considered: blind forgeries, low-force forgeries, and brute-force forgeries. The impact of an incremental level of quality in PAs against signature verification systems was examined, considering off-line and on-line systems using the BiosecurID database.

Overall, the field of Presentation Attack Detection in signature biometrics continues to evolve, with a focus on detecting skilled forgeries and enhancing security against various impostors to ensure reliable signature verification.

2. Materials and Methods

The experimental investigation focused on analyzing Presentation Attack (PA) scenarios in on-line handwritten signature verification [14]. A unique aspect of the research was the consideration of typical PAs in signature verification, where attackers interacted with the sensor in the same manner as a normal access attempt, involving a handwritten signature resembling the targeted identity as seen in Fig 1. The attacker's level of knowledge about the signature played a crucial role in the attack's success rate.

The experimental protocol allowed for the study of both BF and PA scenarios, involving three levels of impostors: (b) random forgeries, (c) static forgeries (trained and blueprint), and (d) dynamic forgeries. Additionally, the case of using the finger as the writing tool in the e-BioSign [20] subset was considered. All available users from the e-BioSign (65 users) and BiosecurID (132 users) subsets were used for evaluation, and no development of the on-line signature verification system was carried out.

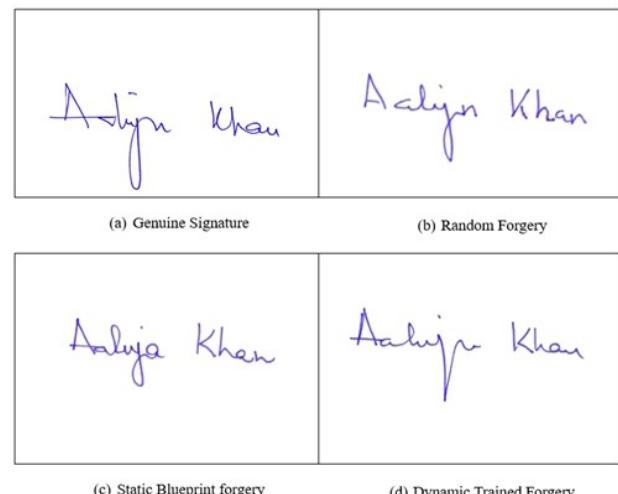


Figure 1: Four signature samples, including one genuine signature and three different types of forgeries, all produced for the same user

3. Results and Discussion

For both databases, genuine signatures from the first session served as reference signatures, and the remaining genuine signatures were used for testing. Skilled forgeries scores (PA mated scores) were obtained by comparing reference signatures against skilled forgeries for each level of attacker, while random forgeries scores (BF non-mated scores) were obtained by comparing reference signatures with genuine signatures from other users. The final score was determined by averaging the four one-to-one comparisons.

The experimental results for the stylus as the writing tool revealed improved system performance for both BiosecurID and e-BioSign databases when the attacker's available information

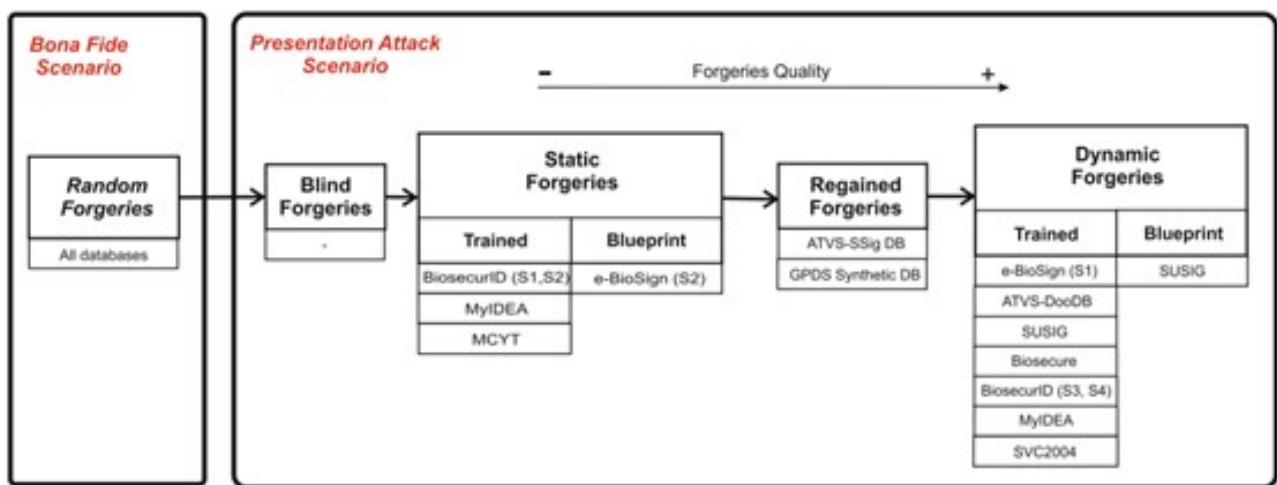


Figure 2: Illustration depicting the various forgery types for both BF and PA scenarios, considering the attacker's available information, training, efforts, and ability to execute the attack.

was reduced. For example, an EER of 7.5% was achieved when the attacker had access to dynamics and static information, whereas it reduced to 5.4% when only static information was provided.

Varying training and effort to perform forgeries had an impact, with higher errors observed in the e-BioSign database for both dynamic and static skilled forgeries compared to the Biosecur ID database. The different scenarios and results for random forgeries (zero-effort impostors) showed similar good performance for both databases. When using the finger as writing tool, a significant degradation in system performance was observed for dynamic forgeries in the e-BioSign database compared to using the stylus. Protecting against potential onlookers while signing on mobile devices could improve results, as skilled forgers might not have access to dynamic information. Additional data captured after e-BioSign achieved a much better EER of 8.9% for dynamic forgeries compared to 18.3% in the original data set. Overall, the study emphasized that the results should be interpreted generally, as specific operational setups can influence outcomes depending on the matching algorithm used. Previous research has shown the efficacy of combining different verification systems to address various types of attacks.

4. Conclusion

In this study, an extensive examination of Presentation Attack (PA) scenarios in on-line handwritten signature verification was conducted. Unlike conventional PAs involving physical artifacts, the typical PAs observed in signature verification entail the attacker mimicking the normal access attempt closely, presenting a handwritten signature that imitates the targeted identity to some extent. The attacker's level of knowledge and its application to the signature being attacked significantly influenced the attack's success rate. The results obtained from both the Biosecur ID and e-BioSign databases revealed substantial

effects on the system's performance, considering not only the attacker's information level but also their training and effort in executing the signature. When using the finger as writing tool, it is recommended for users to safeguard against potential onlookers while signing, especially in mobile scenarios. This precaution can significantly hinder skilled forgers (PA impostors) from accessing dynamic signature information, leading to much better outcomes.

References

- [1] V. Podzorov, E. Menard, A. Borissov, V. Kiryukhin, J. A. Rogers, M. Gershenson, Intrinsic charge transport on the surface of organic semiconductors, *Physical review letters* 93 (8) (2004) 086602.
- [2] F. Balzer, M. Schiek, A. Lützen, K. al-shamery, in: *Proc. SPIE*, Vol. 64706, 2007.
- [3] Y. Luo, M. Brun, P. Rannou, B. Grevin, Growth of rubrene thin film, spherulites and nanowires on sio₂, *physica status solidi (a)* 204 (6) (2007) 1851–1855.
- [4] S. Seo, B.-N. Park, P. G. Evans, Ambipolar rubrene thin film transistors, *Applied physics letters* 88 (23).
- [5] M. C. Scharber, D. Mühlbacher, M. Koppe, P. Denk, C. Waldauf, A. J. Heeger, C. J. Brabec, Design rules for donors in bulk heterojunction solar cells towards 10% energy conversion efficiency, *Advanced materials* 18 (6) (2006) 789–794.
- [6] J. E. Norton, J. r me Cornil, V. Coropceanu, et al., Molecular understanding of organic solar cells: The challenges, *Acc. Chem. Res.* 42 (11) (2009) 1691–1699.
- [7] C. W. Schlenker, M. E. Thompson, The molecular nature of photovoltaic losses in organic solar cells, *Chemical communications* 47 (13) (2011) 3702–3716.
- [8] S.-W. Park, J. M. Hwang, J.-M. Choi, D. Hwang, M. Oh, J. H. Kim, S. Im, Rubrene thin-film transistors with crystalline and amorphous channels, *Applied physics letters* 90 (15).
- [9] J. Galbally, M. Gomez-Barrero, A. Ross, Accuracy evaluation of handwritten signature verification: Rethinking the random-skilled forgeries dichotomy, in: *2017 IEEE international joint conference on biometrics (IJCB)*, IEEE, 2017, pp. 302–310.
- [10] C. Hsu, J. Deng, C. Staddon, P. Beton, Growth front nucleation of rubrene thin films for high mobility organic transistors, *applied physics letters* 91 (19).

- [11] Y. C. Feng, P. C. Yuen, A. K. Jain, A hybrid approach for generating secure and discriminating face template, *IEEE transactions on information forensics and security* 5 (1) (2009) 103–117.
- [12] J. Fierrez, J. Ortega-Garcia, On-line signature verification, in: *Handbook of biometrics*, Springer, 2008, pp. 189–209.
- [13] M. H. M. Yusof, V. K. Madasu, Signature verification and forgery detection system, in: *Proceedings. Student Conference on Research and Development, 2003. SCORED 2003.*, IEEE, 2003, pp. 9–14.
- [14] R. Sanchez-Reillo, Signature analysis in the context of mobile devices, *Image and Vision Computing* 55 (2016) 34–37.
- [15] N. Yager, T. Dunstone, The biometric menagerie, *IEEE transactions on pattern analysis and machine intelligence* 32 (2) (2008) 220–230.
- [16] N. Houmani, S. Garcia-Salicetti, On hunting animals of the biometric menagerie for online signature, *PLoS one* 11 (4) (2016) e0151691.
- [17] S. Dargan, M. Kumar, A comprehensive survey on the biometric recognition systems based on physiological and behavioral modalities, *Expert Systems with Applications* 143 (2020) 113114.
- [18] T. Diserens, J. Bubnicki, E. Schutgens, K. Rokx, R. Kowalczyk, D. Kuijper, M. Churski, Fossoriality in a risky landscape: Badger sett use varies with perceived wolf risk, *Journal of Zoology* 313 (1) (2021) 76–85.
- [19] P. Sharma, M. Kumar, H. Sharma, Comprehensive analyses of image forgery detection methods from traditional to deep learning approaches: an evaluation, *Multimedia Tools and Applications* 82 (12) (2023) 18117–18150.
- [20] P. Craddock, *Scientific investigation of copies, fakes and forgeries*, Routledge, 2009.

RESEARCH PROSPECTS IN NATURAL SCIENCES IS A BIENNIAL SCHOLARLY PERIODICAL ISSUED BY GOVT GRADUATE COLLEGE TOWNSHIP, LAHORE, THAT STRIVES TO DISSEMINATE THE EMPIRICAL WORK OF RESEARCHERS WORLDWIDE THROUGH SCRUTINIZED RESEARCH ARTICLES, WITH THE INTENTION OF ESTABLISHING ITSELF AS A LEADING FORUM FOR TOP-NOTCH RESEARCH PUBLICATIONS AND ENDORSING LEARNED TREATISES AND RESEARCH PAPERS.

