INDEXING

Crossref

ORCID
Connecting Research and Researchers

OPEN ACCESS

Google Scholar

publons

DRJI
Directory of Research Journal Indexing

Scilit

EuroPub
Directory of Academic and Scientific Journals

OCLC WorldCat®

sciforum

Semantic Scholar

BASE

ASIAN Research Index

PASTIC
Pakistan Scientific & Technological Information Centre

IMEMR
INDEX MEDICIUS
FOR THE EASTERN MEDITERRANEAN REGION

World Health Organization
Regional Office for the Eastern Mediterranean

ICMJE
INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

LOCKSS

Aims and Scope

Pakistan Biomedical Journal is an open access, peer-reviewed International journal that publishes in all fields of health, allied health and biomedical sciences for an internationally diverse authorship. Clinical studies, clinical trials, population studies, public health, discovery medicine, study of microbes, molecular and cellular biology, basic mechanisms of biology, genetics, cancer biology, molecular medicine, pharmacology, virology, chemical biology, immunobiology, chemical biology, physiological and pathological studies are within the scope of journal.

A highly-cited, multi disciplinary, international editorial board assures timely publication of manuscripts and reviews on latest advancements in biomedical sciences.

Types of Articles

- Research papers
- Short communications
- Review or mini-reviews
- Commentaries
- Perspectives, opinion
- Meta-analysis
- Case reports
- Case studies
- Case-control studies

Reviews on recent progress in biomedical sciences are commissioned by the editors. The purpose of the Pakistan Biomedical Journal is to publish scientific and technical research papers to bring attention of international researchers, scientists, academicians, health care professionals towards the recent advancements in biomedical sciences. The articles are collected in the form of reviews, original and clinical studies. It may serve as a global platform for scientists in relevant fields to connect and share ideas mutually. This journal is open to all the research professionals whose work fall within our scope. Submission are welcome and may be submitted here.

submissions@pakistanbmj.com

@JournalPakistan
@Pakistanbmj

Title
The title of the paper should provide a concise statement of the contents of the paper. A good title is very important and will attract readers and facilitate retrieval by online searches, thereby helping to maximize citations. The title should include topical keywords and allude to the interesting conclusions of the paper. A title that emphasizes the main conclusions, or poses a question, has more impact than one that just describes the nature of the study.

Running Head
Running head should be added in the header along with the page numbers.

Type of Article
Research Article/ Case Report/ Review Article/ Opinion/ Short Communication/ Mini Review/ Letter to Editor

Running Title: A short version of the paper title.
Keywords: The major keywords used in the article have to be mentioned.

Authors
List here all author names Author[1], Author[2] and Author[3]
[1]Author department, University, Country
[2]Author department, University, Country
[3]Author department, University, Country

*Corresponding Author
Author name, Affiliation, Department Name, University Name, Address, City, State, Country, E-mail:

Abstract
Abstract should include a brief content of the article. It should be structured not more than 250 words. It should include following sub headings: Objective, Methods, Results, Conclusions.

Abbreviations
If there are any abbreviations in the article they have to be mentioned.

INTRODUCTION
Provide a context or background for the study (i.e.,the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be made clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

METHODS
The Methods section should include only information that was available at the time the or plan of the protocol. All information gathered during the conduct of study should be included in the result section.
Study Design, Inclusion / Exclusion Criteria, Data collection procedure, Statistical analysis.

RESULTS
Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first.

Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. When data are summarized in the results section, give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical methods used to analyze them. Table font should be 10 and caption should be below table and figure.

Data should not be duplicated in both figures and tables. The maximum limit of tables and figures should not exceed more than 4. Mention the findings of the study in paragraph, while mentioning figure and table number in text in sequential order.

TABLE

Table should not be copy pasted or in picture form

DISCUSSION

Discuss your findings by comparing your results with other literature

REFERENCES

References should not be less than 20.
In text references should be in number style. For Example [1]
Follow the Pubmed Referencing style
Provide the DOI link

Example: Cook NR, Rosner BA, Hankinson SE, Colditz GA. Mammographic screening and risk factors for breast cancer. American Journal of Epidemiology. 2009 Dec; 170(11): 1422-32. doi: 10.1093/aje/kwp304.

If there are more than six authors, write *et al*. after the first six names.

CONCLUSION(S)

Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

Provide the list of individuals who contributed in the work and grant details where applicable

Plagiarism policy

Similarity index should be less than 19 and less than 5 from individual sources.

Authorship Letter

Signed authorship letter by all authors including there current department, University, City, Country, Email.

Declaration Form

Signed declaration form submit by corresponding author.

The submission of article should include: manuscript according to journal guidelines, authorship letter, declaration form. It should be submitted to the following email id:

submissions@pakistanbmj.com

EDITORIAL TEAM

VOLUME 06 ISSUE 03

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

EDITORIAL BOARD

VOLUME 06 ISSUE 03

Advisory Board

Prof. Dr. Shagufta Naz, Ph.D.
Lahore College for Women University, Lahore, Pakistan

Prof. Dr. Farkhanda Manzoor, Ph.D.
Lahore College for Women University, Lahore, Pakistan

Prof. Dr. Nadeem Sheikh ,Ph.D.
University of the Punjab, Lahore, Pakistan

Prof. Dr. Muhammad Saleem Rana, Ph.D.
The University of Lahore, Lahore, Pakistan

National Members

Dr. Munir Bhinder, Ph.D.
Associate Professor
University of Health Sciences, Lahore, Pakistan

Dr. Fareeha Hameed , Ph.D.
Associate Professor
Forman Christian College, Lahore, Pakistan

Dr. Maham Akhlaq, MBBS, M.Phil, DipRCpath, Ph.D.
Assistant Professor
University of Health Sciences, Lahore, Pakistan

Dr. HA Raza, Ph.D.
University of Agriculture, Faisalabad, Pakistan

Dr. Sami Ullah Mumtaz, MBBS, FCPS
Assistant Professor
Mayo Hospital, Lahore, Pakistan

Dr. Kulsoom Rahim, Ph.D.
Assistant Professor
University of Engineering and Technology, Texila, Pakistan

Dr. Shafqat Ali, Ph.D.
Assistant Professor
Ghulam Ishaq Khan University, Swabi, Pakistan

International Members

Dr. Diki, M.ED., Ph.D.
University Terbuka, Indonesia

Dr. Beatrice Paradiso, MD, Double Ph.D.
Dolo Hospital, Venice, Italy

Dr. Rizwan Ullah Khan, MBBS, FCPS
Associate Professor
King Fahad Specialist Hospital, Jeddah, Saudi Arabia

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

TABLE OF

CONTENTS

VOLUME 06 ISSUE 03

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

DOI: https://doi.org/10.54393/pbmj.v6i3.857

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
Volume 6, Issue 3 (March 2023)

Prevalence of Non-Communicable Diseases

Ahmed Alwazzan[1*]

[1]Division of Gynecology Oncology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
Ab_wazzan@yahoo.com

ARTICLE INFO

How to Cite:

Alwazzan, A. . (2023). Prevalence of Non-Communicable Diseases . Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.857

Non-communicable diseases (NCDs) are a growing health concern in Pakistan, with a significant impact on the country's population and economy. NCDs are chronic conditions that are not caused by infectious agents and are generally linked to lifestyle factors, such as unhealthy diets, lack of physical activity, and tobacco and alcohol use. The prevalence of NCDs in Pakistan has increased rapidly over the past few decades, with estimates suggesting that more than 60% of deaths in the country are now attributed to NCDs. The most common NCDs in Pakistan are cardiovascular diseases, diabetes, cancer, and chronic respiratory diseases. These conditions not only have a devastating impact on individuals and families, but also place a significant burden on the healthcare system and the economy. There are several reasons why NCDs are on the rise in Pakistan. One of the key factors is the changing lifestyle patterns in the country, with increasing urbanization, sedentary lifestyles, and unhealthy diets. Poverty, lack of education, and poor healthcare infrastructure are also contributing factors, as they limit access to healthy foods, physical activity, and medical care. To address the growing prevalence of NCDs in Pakistan, there is an urgent need for a coordinated and comprehensive approach. This should involve a range of stakeholders, including the government, healthcare professionals, civil society organizations, and the private sector. One of the key interventions that can be implemented is to promote healthy lifestyles and prevent risk factors for NCDs. This includes initiatives to improve access to healthy foods, promote physical activity, and reduce tobacco and alcohol use. Health education and awareness campaigns can also play a critical role in raising awareness of the risks associated with NCDs and promoting healthy behaviors. Another critical intervention is to strengthen healthcare systems to ensure that NCDs are diagnosed and treated early. This requires investments in healthcare infrastructure, including training healthcare professionals and improving access to medical equipment and supplies. It also involves developing effective referral systems to ensure that patients receive appropriate care and treatment. Finally, there is a need for research and innovation to better understand the causes and mechanisms of NCDs in Pakistan. This includes developing locally relevant research studies and using the findings to inform policy and practice. In conclusion, the prevalence of NCDs in Pakistan is a significant public health concern that requires urgent attention. By adopting a comprehensive approach that addresses both prevention and treatment, and by engaging a range of stakeholders, it is possible to mitigate the impact of NCDs and improve the health and wellbeing of the Pakistani population.

REFERENCES

[1] Khan E, Prakoso D, Imtiaz K, Malik F, Farooqi JQ, Long MT, et al. The clinical features of co-circulating dengue viruses and the absence of dengue hemorrhagic fever in Pakistan. Frontiers in public health. 2020 Jun 17;8. doi: 10.3389/fpubh.2020.00287 Pattern of dois should be consistent

[2] Umair M, Haider SA, Rehman Z, Jamal Z, Ali Q, Hakim R, et al. Genomic Characterization of Dengue Virus Outbreak in 2022 from Pakistan. Vaccines. 2023 Jan;11(1). doi: 10.3390/vaccines11010163

DOI: https://doi.org/10.54393/pbmj.v6i3.855

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 3 (March 2023)

Review Article

Current Applications and Future Perspective of CRISPR/Cas9 in the Diagnosis and Treatment of COVID 19: A Review

Amna Mahmood[1*], Malaika Ajaz[2], Waleed Rasool[2], Maleeha Manzoor[1] and Nida Naeem[2]

[1]Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[2]Department of Biotechnology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan

ARTICLE INFO

Key Words:

Clustered-Regularly Interspaced Short Palindromic Repeats (CRISPR), CRISPR Associated Protein (Cas9), Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-Cov-2), COVID-19, Genetic Engineering

How to Cite:

Mahmood, A. ., Ajaz, M. ., Rasool, W. ., Manzoor, M. ., & Naeem, N. . (2023). Current Applications and Future Perspective of CRISPR/Cas9 in the Diagnosis and Treatment of COVID 19: A Review: CRISPR/Cas9 in the Diagnosis and Treatment of COVID 19. Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.855

***Corresponding Author:**

Amna Mahmood
Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
amnaqureshi10@hotmail.com

Received Date: 4th March, 2023
Acceptance Date: 25th March, 2023
Published Date: 31st March, 2023

ABSTRACT

Since the outbreak of COVID-19, scientists have applied various techniques to diagnose and treat the viral disease. However, due to the limitations of other methods, they deployed Clustered-Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) protein (CRISPR/Cas) system that not just successfully diagnosed but also facilitated the therapeutic treatment of the COVID-19. CRISPR-Cas9 was first identified in the bacteria E. coli, which has a unique immune system for cutting the nucleic structures of invasive species. Scientists studied the bacterial system that led to the development of an identical model, generally called the CRISPR-Cas9 genome editing system. It has a guide RNA (gRNA) and Cas9 proteins; gRNA identifies and leads cas9 protein to cleave the specific sequence. This technique has dynamic applications, such as the ability to correct mutations by cleaving the mutant cells and to detect and develop optimal treatments for viral diseases like severe acute respiratory syndrome coronavirus-2 (SARS-CoV2). Apart from the extensive advantages of CRISPR-Cas technology, there are serious concerns regarding the commercialization of this technique. A rational suggestion would be to use it to resist a pandemic like COVID-19 rather than triggering another human race of genome enhancement. This article is aimed to review the background of CRISPR-Cas9, its mechanism as a diagnostic and therapeutic tool for COVID-19, whereas its limitations, future aspects, and ethical boundaries are discussed subsequently.

INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2) has contracted to almost 80 million people around the globe while nearly 2 million people have died since December 2019 when it was first discovered in Wuhan province of China. The disease was spreading so rapidly that millions were getting infected in a single day and the R0 Number (number of new individuals infected by the already infected individual) of coronavirus was estimated to be three [1]. Therefore, there was much need for authentic diagnostic and therapeutic tools to control the disease, reduce the risk of transmission and save the lives of infected people through medications or vaccinations [2]. Hitherto, various conventional diagnostics techniques such as sequencing based methods, immunological methods and PCR based techniques were deployed to detect the novel coronavirus, yet, due to limitations of these methods and low accuracy, it was critical to find methods that give higher accuracy and quick results. In this regard, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-Associated System CAS – a versatile gene editing tool is deployed to facilitate diagnosis and treatment of

DOI: https://doi.org/10.54393/pbmj.v6i3.855

SARS-CoV-2. CRISPR-Cas structures were first found in E.coli bacteria back in the 1980s that guard cells from the foreign invasion species [3]. Since the invention of CRISPR-CAS9 genome editing technology biomedical research has been revolutionized [4]. The technology can perform gene edits of three critical categories which includes disruption, deletion and insertion or correction to treat diseases. This novel genetic editing system targets the abnormal proteins in the DNA, cuts or modifies the abnormal proteins and lets them repair by the natural DNA repairing mechanism [3]. The lexicon of CRISPR technology is that it comprises two parts – an associated enzyme called 'Cas9' and a 'Guide RNA'. Both combine to form a complex known as Cas9/Guide RNA complex – while Cas9 acting as 'molecular scissors' to cut the DNA and guide RNA leads Cas9 to target specific locations in DNA (Figure 1).

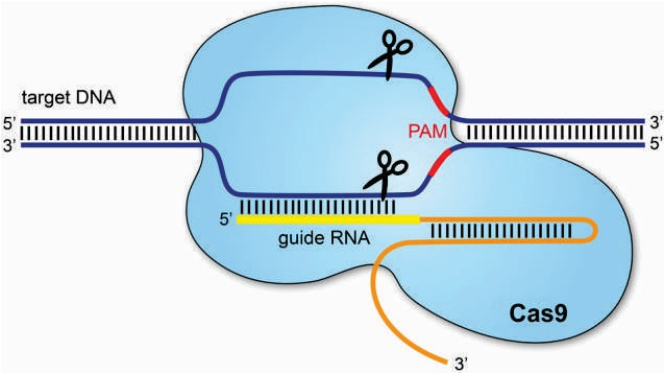

Figure 1: CRISPR-Cas9[5]

The technology has brought promising results in diagnosis and treatment of viral diseases – hepatitis virus infections, HIV, rabies, Dengue etc. Not just infectious diseases, it has provided accuracy in determining hereditary diseases like β-thalassemia, hematologic diseases like sickle cell anemia (SCA). On the other hand, it has been applied in a variety of other species including drosophila, mouse, rat, bacteria and yeast [6]. Due to its high efficiency and low cost, CRISPR-Cas systems have immense applications ranging from disease research and diagnosis, genome-scale screening to gene therapies. 1 However, this review article is primarily focused on the applications of the CRISPR-Cas systems for the diagnosis and therapeutics of SARS-CoV-2, the challenges and limitations associated with the system.

CRISPR-Cas9 as a diagnostic tool for COVID-19

Prevention and rapid treatment of the disease needs quick detection of disease-causing agents. For rapid diagnostic purposes, traditional and conventional techniques including antigen testing, restriction enzymes based, PCR-based methods, and isothermal amplification-based, sequencing-based techniques were previously used. Though these techniques have limitations that make them unable to meet the modern era sensitivity needs of pathogen detection, for instance, detection of different viruses like dengue and SARS-CoV-2 [7]. Compared to these techniques, CRISPR-CAS is applied for pathogen detection which is a highly specific, precise and sensitive technique. It is a next generation technique that can detect the disease-causing pathogens even highly variant viruses in comparatively less time and with better efficiency. Several studies and research have shown the potential of the CRISPR-CAS method as a diagnostic tool for SARS-CoV-2 (COVID 19) virus and various bacterial infections (4). It is one of the best genomes editing tools, consisting of two main classes based on presence of Cas protein i-e, CRISPR Cas Class 1 (types I/cas3, III/cas10, and IV) and CRISPR Cas class 2 (II/Cas9, V/Cas12, and VI/Cas13). CRISPR-CAS9 that facilitated the development of the antiviral strategies for SAR-CoV-2 belongs to class 1 [8]. It works with single subunit Cas protein unlike the first category, which uses multiple subunit proteins for cleavage and targeting the specific sequence. Cas 9 and 12 type target double stranded DNA and Cas 13 targets single stranded RNA.

Mechanism

After determining the desired sequence in DNA, nuclease activity of Cas protein comes forward to take action and targets the point in the sample. These nucleases mediated degradation is tagged with fluorophore dye. The dye produces fluorescent signals that are used as indicators for the determination of a specific sequence which is the main target. Different techniques based on CRISPR Cas 9 mainly Cas 9 and Cas 13 are applied for SARS-CoV-2 detection including SHERLOCK assay (CRISPR Cas 13 based), FELUDA assay (Cas 9 based), detector assay (Cas 12a based), CONAN assay (Cas 3), VaNGuard assay (Cas 12a enzyme-based) etc. SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking), however, is specially developed for the detection of COVID-19 [1]. CRISPR Cas 9 based enzymatic technique for the detection of COVID is FELUDA assay which is considered as a common technique for detection of viral infections. Due to its magnificent diagnostic capability of COVID virus, it is named as FnCas9 or FELUDA assay. This technique consists of basically three steps. First, the RNA sample is taken from a specimen. As COVID virus is RNA virus, so RNA sample is considered. This RNA is converted to cDNA by the activity of reverse transcriptase and biotin-labeled primer is amplified for detection. Then FnCas 9 complex is added to it which is prepared by combination of FnCas enzyme, FAM labeled transcRNA and sgRNA. Ribonucleoprotein (RNP) is bound to the target sequence that activates the FnCas complex. This will result in FAM labeled transcRNA cleavage. In the last, gold nanoparticles conjugated with

DOI: https://doi.org/10.54393/pbmj.v6i3.855

RAM antibodies are added for precise detection (Figure 2) [6].

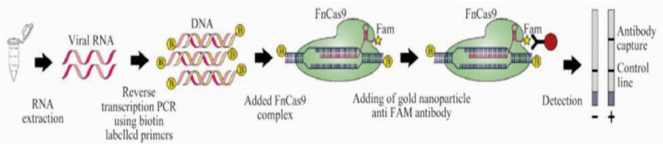

Figure 2: Schematic layout of CRISPR Cas 9 based COVID 19 detection[6]

CRISPR-Cas9 as a therapeutic tool for COVID-19

In a short period since its discovery, CRISPR has successfully built a massive impact on the field of scientific research [9]. Its genome editing function grasps the attention of scientists to find significant applications in numerous disciplines like biodefense, best-quality food production, and fetal medicine production. It can be effectively used to treat infectious viral and bacterial diseases. Comprehensive research has been done and thousands of publications have been written on its incredible efficiency – how it can diagnose diseases in early-stages and treat various infectious diseases like cancer and tuberculosis (TB) [10]. During the COVID-19 outbreak, CRISPR-Cas systems came as a potential diagnostic and therapeutic tool – mainly its class Cas-9 and Cas-13 are considered a preferred option to treat and diagnose SARS-CoV-2 [11]. With the help of a guide-RNA array, its small size makes it appropriate for an "all-in-one" AAV (Adeno-associated virus) to target COVID RNA viruses with high specificity. It cuts the complementary RNA sequence, which is the main target. Unlike Cas 13, Cas 9 needs PAM (protospacer motif) to target the single-stranded RNA sequence (ssRNA). Once the target point is recognized, this COVID incorporated segment is cleaved and changed with the healthy DNA variant [12].

Mechanism of CRISPR-Cas9

CRISPR-Cas9 system includes CRISPR-associated proteins or Cas proteins and gRNA (guide RNA). Cas 9 protein, also known as a genomic scissor, is extracted from the bacterium (Streptococcus pyogenes) and is responsible for the cleavage of DNA strands [13]. Furthermore, two lobes, including the NUC (nuclease) lobe and REC (recognition) lobe, are two regions of the Cas-9 protein. Similarly, crRNA (CRISPR RNA) and transcRNA (trans-activating CRISPR RNA) are two parts of gRNA. It is primarily a three steps process; recognition, breakage, and repair. Through crRNA complementary base pair sequence, Cas 9 protein recognizes the gene of interest (infected area by SARS-CoV-2) directed by sgRNA [14]. Cas 9 protein cannot work until and unless sgRNA is present. It then breaks the double-stranded helix at the upstream region of PAM. DNA starts melting, followed by DNA-RNA hybrid formation and RuvC domains cut the complementary and non-complementary strands, respectively. The infected region is cleaved, followed by a DNA repairing mechanism to make it a healthy variant. NHEJ (Non-Homologous End Joining) and HDR (Homology-directed Repair) are commonly used techniques for repairing processes in the CRISPR Cas 9 system (Figure 3) [15].

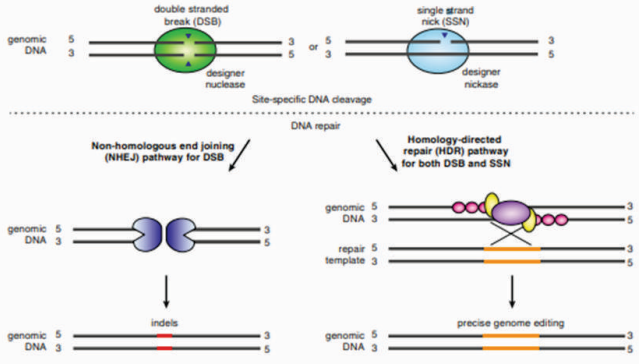

Figure 3: Mechanism of CRSIPR Cas 9 to treat infectious disease like COVID 19 [16].

Limitations

Besides numerous applications of precision, sensitivity, better genome editing tool, working in less time, and role in saving millions of lives, CRSIPR-Cas 9 has some limitations as well which opens the window of the latest CRSIPR systems. It can cause genomic loss while working at target sequence as it is not 100 percent accurate. It is not flexible and specific, i.e., cannot be applied in any sequence of viral genome. It requires PAM to target the sequence at the editing site and only cleave RNA directly. It can cleave DNA after the activity of reverse transcriptase enzymes [12]. CRSIPR Cas technique is efficient, inexpensive and exact but it requires highly experienced staff and strong legislation to be practical. Ethical decisions are made to apply this technology to evaluate potential risk-benefit ratio [17]. So that benefits are maximized and risks are minimized. It can edit the genome of gametes or germ line cells in addition to somatic cells (germline editing). It not only modifies the individual but also his progeny and that should be kept under limits and ethics. Besides curing different abnormalities and mutations, it can enhance the desirable features. So scientists and researchers abide by this technique under moratorium and official policies made for human genome editing [10].

Future Aspects and Ethics

It is clear from the above discussion that the CRSIPR-Cas system possesses the potential to correct mutations, hereditary diseases, fight viral diseases and more. The adoption of this tool has brought beneficial results for the scientific community and has facilitated modern biotechnology [18]. It is because of such advancements

DOI: https://doi.org/10.54393/pbmj.v6i3.855

that has led biotechnology to genetically engineer plants, microorganisms and animals in a much easier way. Regarding the future of CRSIPR, it is suggested that the government, scientific community and researchers must pay attention to its availability. On the other hand, the world is also witnessing the rise of pharmacogenomics. Further studies found that CRSIPR-Cas9 can directly penetrate the genomic sequence of the cell by employing 'cell-penetrating peptides' or 'nucleofection' that will allow quick editing. This genome editing technology will pave the way forward for designing such drugs that could treat a vast range of diseases and mutations – and certainly equip the scientists, healthcare workers, and researchers to prepare for pandemics like COVID-19 beforehand [19]. Besides, its potential power to treat diseases – there is a serious impediment in the progress and implementation of CRSIPR which is 'gene enhancement' or 'gene editing' of human beings. Or more simply put it can be used for unethical purposes, thereby introduction of it in clinical practice would certainly raise social concerns. In the past scientists have made attempts on human embryos using CRSIPR technology to knock out abnormal genes such as the CCR5 gene responsible for HIV virus which resulted in criticism from the scientific communities [20]. The fact that this technology can bring heritable genetic traits can cause a race of 'designer babies' or even worse such as wild or lawless modifications of human beings that can lead to extinction of entire species [21]. Not only humans, it can induce variations in other animals as well and as we have learned from the COVID-19 outbreak – a little mutation can bring the whole world on its knees. For that reason, it is mandatory that scientific communities, governments and legislative bodies must set or define its boundaries – there must be a clear distinction between the genetic treatment and genetic enhancement. Henceforth, techniques like CRSIPR could not get involved in heinous misdeeds and the field of science can progress [10].

CONCLUSIONS

CRSIPR genome editing technology undoubtedly holds a promising future for treating and diagnosing diseases. The two components of CRSIPR-cas9, i.e., Cas9 effector proteins and Guide RNA, are crucial for the future of genome engineering as they allow the modification of DNA sequences. Furthermore, there is room for improvement in delivering Cas9 and its associated guide RNA to target specific sequences of cells. The newly evolving Cas9 enzymes from the bacteria, such as the *Streptococcus pyogenes* type II CRSIPR system, will significantly enhance the delivery method to cleave desired sequences. CRSIPR-Cas9 has played a substantial role in fighting the recent COVID-19 pandemic. It has proved its ability to diagnose, genetically edit and treat viral diseases, genetic disorders, and tumors. On the other hand, this system's powerful genome editing ability has raised social and ethical concerns for its commercialization. The applications of this technology can trigger a race for an enhanced genome that will lead to a superhuman race or even worse. Consequently, scientists and society at large have to address the commercial applications of this technique so that maximum benefits could reap out of it while minimizing the risk factors.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Libman MD. Correspondence letter regarding 'is standby therapy for COVID-19 a practical option for travellers? Journal of Travel Medicine. 2023 Apr: taad042. doi: 10.1093/jtm/taad042.

[2] Park SE. Epidemiology, virology, and clinical features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; coronavirus disease-19). Pediatric Infection and Vaccine. 2020 Apr; 27(1): 1-10. doi: 10.14776/piv.2020.27.e9.

[3] Waris A, Atta UK, Ali M, Asmat A, Baset AJ. COVID-19 outbreak: current scenario of Pakistan. New Microbes and New Infections. 2020 May; 35: 100681. doi: 10.1016/j.nmni.2020.100681.

[4] Chan KG, Ang GY, Yu CY, Yean CY. Harnessing CRSIPR-Cas to combat COVID-19: From diagnostics to therapeutics. Life. 2021 Nov; 11(11): 1210. doi: 10.3390/life11111210.

[5] Redman M, King A, Watson C, King D. What is CRSIPR/Cas9? Archives of Disease in Childhood-Education and Practice. 2016 Aug; 101(4): 213-5. doi: 10.1136/archdischild-2016-310459.

[6] He X and Zeng XX. Immunotherapy and CRSIPR Cas Systems: Potential Cure of COVID-19? Drug Design, Development and Therapy. 2022 Mar; 16: 951-72. doi: 10.2147/DDDT.S347297.

[7] Rajan A, Shrivastava S, Kumar A, Singh AK, Arora PK. CRSIPR-Cas system: from diagnostic tool to potential antiviral treatment. Applied Microbiology and Biotechnology. 2022 Sep; 106(18): 5863-77. doi: 10.1007/s00253-022-12135-2.

[8] Ebrahimi S, Khanbabaei H, Abbasi S, Fani M, Soltani S, Zandi M, Najafimemar Z. CRSIPR-Cas system: A promising diagnostic tool for COVID-19. Avicenna Journal of Medical Biotechnology. 2022 Jan; 14(1): 3-

DOI: https://doi.org/10.54393/pbmj.v6i3.855

9. doi: 10.18502/ajmb.v14i1.8165.
[9] Li Y, Liang J, Deng B, Jiang Y, Zhu J, Chen L, *et al.* Applications and Prospects of CRSIPR/Cas9-Mediated Base Editing in Plant Breeding. Current Issues in Molecular Biology. 2023 Feb; 45(2): 918-35. doi: 10.3390/cimb45020059.
[10] Chekani-Azar S, Gharib Mombeni E, Birhan M, Yousefi M. CRSIPR/Cas9 gene editing technology and its application to the coronavirus disease (COVID-19), a review. Journal of Life Science and Biomedicine. 2020 Jan; 10(1): 1-9. doi: 10.29252/scil.2020.jlsb1.
[11] Hernandez-Garcia A, Morales-Moreno MD, Valdés-Galindo EG, Jimenez-Nieto EP, Quezada A. Diagnostics of COVID-19 Based on CRSIPR–Cas Coupled to Isothermal Amplification: A Comparative Analysis and Update. Diagnostics. 2022 Jun; 12(6): 1434. doi: 10.3390/diagnostics12061434.
[12] Lotfi M and Rezaei N. CRSIPR/Cas13: A potential therapeutic option of COVID-19. Biomedicine & Pharmacotherapy. 2020 Nov; 131: 110738. doi: 10.1016/j.biopha.2020.110738.
[13] Sun Q, Wang Y, Dong N, Shen L, Zhou H, Hu Y, *et al.* Application of CRSIPR/Cas9-based genome editing in studying the mechanism of pandrug resistance in Klebsiella pneumoniae. Antimicrobial Agents and Chemotherapy. 2019 Jul; 63(7): e00113-19. doi: 10.1128/AAC.00113-19.
[14] Hillary VE and Ceasar SA. A Review on the Mechanism and Applications of CRSIPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. Molecular Biotechnology. 2023 Mar; 65(3): 311-25. doi: 10.1007/s12033-022-00567-0.
[15] Asmamaw M and Zawdie B. Mechanism and Applications of CRSIPR/Cas-9-Mediated Genome Editing. Biologics. 2021 Aug; 15: 353-361. doi: 10.2147/BTT.S326422.
[16] Devaraju N, Rajendiran V, Ravi NS, Mohankumar KM. Genome Engineering of Hematopoietic Stem Cells Using CRSIPR/Cas9 System. Stem Cell Assays: Methods in Molecular Biology. 2022 May; 2429: 307-331. doi: 10.1007/978-1-0716-1979-7_20.
[17] Nafian F, Nafian S, Kamali Doust Azad B, Hashemi M. CRSIPR-based diagnostics and microfluidics for COVID-19 point-of-care testing: a review of main applications. Molecular Biotechnology. 2023 Apr; 65(4): 497-508. doi: 10.1007/s12033-022-00570-5.
[18] Li PY, Li SQ, Gao SG, Dong DY. A one-step platform for screening high-efficient and minimal off-target CRSIPR/Cas13 crRNAs to eradicate SARS-CoV-2 virus for treatment of COVID-19 patients. Medical Hypotheses. 2022 Feb; 159: 110754. doi: 10.1016/j.mehy.2021.110754.
[19] Ding R, Long J, Yuan M, Jin Y, Yang H, Chen M, *et al.* CRSIPR/Cas system: a potential technology for the prevention and control of COVID-19 and emerging infectious diseases. Frontiers in Cellular and Infection Microbiology. 2021 Apr; 11: 639108. doi: 10.3389/fcimb.2021.639108.
[20] Bhattacharjee G, Gohil N, Khambhati K, Mani I, Maurya R, Karapurkar JK, *et al.* Current approaches in CRSIPR-Cas9 mediated gene editing for biomedical and therapeutic applications. Journal of Controlled Release. 2022 Feb; 343: 703-23. doi: 10.1016/j.jconrel.2022.02.005.
[21] Gonzalez MN, Massa GA, Andersson M, Storani L, Olsson N, Decima Oneto CA, *et al.* CRSIPR/Cas9 Technology for Potato Functional Genomics and Breeding. Methods in Molecular Biology. 2023 Mar; 2653: 333-61. doi: 10.1007/978-1-0716-3131-7_21.

DOI: https://doi.org/10.54393/pbmj.v6i3.854

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 3 (March 2023)

Original Article

A Comprehensive Study on *Asparagus officinalis*: Its Antimicrobial, Antioxidant and Phytochemical Characteristics

Add and before the name of the last author

Amna Mahmood[1*], Malaika Ajaz[2], Waleed Rasool[2], Lahraseb Khan[3], Nida Naeem[2], Ali Hassan[2]

[1]Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[2]Department of Biotechnology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[3]Department of Biochemistry, The University of Azad Jamu and Kashmir, Azad Kashmir, Pakistan

ARTICLE INFO

Key Words:
Asparagus officinalis, DPPH, Phytochemicals, Antioxidants

How to Cite:
Mehmood, A. ., Ajaz, M. ., Rasool, W. ., Khan, L. ., Naeem, N. ., & Hassan, A. . (2023). A Comprehensive Study on Asparagus officinalis: Its Antimicrobial, Antioxidant and Phytochemical Characteristics : Antimicrobial, Antioxidant and Phytochemical Characteristics of Asparagus officinalis. Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.854

***Corresponding Author:**
Amna Mahmood
Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
amnaqureshi10@hotmail.com

Received Date: 28th February, 2023
Acceptance Date: 26th March, 2023
Published Date: 31st March, 2023

ABSTRACT

The Asparagus plant is considered to be a palatable chemical source against treating infectious diseases and flavorings. Its prevalent distribution is well-known in Asian and sub-Asian regions. **Objective:** To understand different activities that have been functional in the stem and leaf extracts *of Asparagus officinalis* including antioxidant and antibacterial activities. Further, phytochemical constituents of asparagus are also discussed. **Methods:** The antibacterial assay of extracts for the variety of bacteria, indicated a maximum inhibition zone against *Enterococcus faecalis* (ATCC 29212) (24 mm) followed by *Staphylococcus aureus* (ATCC 25923) (34 mm), whereas *Bacillus subtilis* (ATCC 6633) (14 mm) at their respective temperature a minimum inhibition zone after 24 hours and 48 hours of incubation (37 °C for bacteria). **Results:** As a robust antioxidant reference standard, these antioxidant activities resulted in the stable radical 1-diphenyl-2-picrylhydrazyl (DPPH). It can be reduced to yellow-coloured DPPH-H, reaching 75.81% of the DPPH scavenging impact at its 100% concentration in contrast to ascorbic acid. Various experiments have been carried out, including the Molisch test, Ninhydrin test, Wagner's test, Alkaline reagent test, Froth test, Ferric reagent test, and Salkowski test for the phytochemical analysis. **Conclusion:** To sum that up, carbohydrates, saponins, and flavonoids are present in these extracts. These extracts were found to perform satisfactory activities in all tests.

INTRODUCTION

Asparagus officinalis (Green Asparagus) is of utmost importance and has worldwide consumption – mainly because of its rich bioactive and nutritional composition. These bioactive contents include inosine, caffeic acid, ferulic acid, rutin and quercetin [1]. Due to their benefits in different ecosystems, like the provision of fuel, medicine, shelter, food, condiments, perfumes, or aromas, it plays an integral role in global sustainability [2]. Plants constitute the primary life-sustaining system by forming soft green protective layers around the earth. As hydrological preservation units and animal food sources are essential for maintaining atmospheric temperature and equilibrium. The average cultivation life-cycle of *A. officinalis* is up to 40 years, as long as their productivity and quality persist. The growth of asparagus deteriorates as time passes due to different diseases invasion by microorganisms, such as *Phytophthora*, *Fusarium*, *Phomopsis asparagi*, *Stemphylium*, and *Cercospora asparagi Sacc* species. Not to mention that asparagus breed auto toxins and indulge in self-harming, thereby affecting the growth of new asparagus plants [3]. Consequently, it becomes indispensable to regenerate the rootstock to overcome the growth barrier. Moreover, in many countries, including New Zealand, the mature asparagus roots are discarded or flung away in barren lands – which is unproductive practice [4]. Because, the debris of asparagus is reportedly claimed as a

DOI: https://doi.org/10.54393/pbmj.v6i3.854

potential source for bioactive compounds such as polyphenols, saponins, and flavonoids. The Agar dilution method was deployed to determine the antibacterial activity of extracts. At the same time, antioxidant properties were assessed using the '2,2-diphenyl-1-picrylhydrazyl' (DPPH) assay. The results found that the minimum inhibitory concentration (MIC) of the leaf is at 0.125 mg/mL concentration against *S. saprophyticus and E.cloacae*, and 1 mg/mL against *S. aureus* and *B.subtilis* [5]. However, no MIC was found in the stem extract at any given concentration. The stem extract activity has shown very low free radical scavenging activity, though the leaf extract accrued effective activity (72.1%). Additionally, qualitative phytochemical scrutiny of these plant extracts confirmed the presence of various chemicals including saponins, tannins, flavonoids, and phlobatannins [6]. Since applying one antimicrobial and antioxidant technique may not provide efficient results, using antimicrobial analysis techniques in more than one concentration is recommended (25mg, 50mg, 75mg, and 100mg). Besides, the results of the antioxidant analysis demonstrate that in-vivo antioxidant capacity is higher than A. *officinalis*, is a callus tissue, and grows in-vitro. Furthermore, antioxidant numbers in vivo *A. officinalis* are less than in vitro-grown ones. The 'antimutagenic agents' that provide protection against mutations and impede the production of cancerous cells (that often lead to the early stage of the disease) are *Beltsville Area*. The removal of xenobiotic and carcinogenic compounds is facilitated by detoxifying enzymes of category cellular phase II – they are also supporting factors of many liver functions [7]. Enhanced antioxidant activity such as cyclooxygenase-2 suppression (less chronic inflammation) can significantly promote a strong immune system and healthier digestion.

METHODS

The materials (plants) used in this analysis were collected from different areas of Lahore and Karachi. The asparagus plant (leaves) was dried and dissolved in ethanol – filtered afterwards. A rotary evaporator was used to separate solvent and extract; then using falcon tubes, it was dried in a water bath. Later, the dried extract was left to freeze for further analysis [8]. A total of five bacteria were selected to conduct the research study. Gram-positive *Staphylococcus aureus* (ATCC 25923), and five Gram-negative *Pseudomonas Aeruginosa* (ATCC 27853), *B. subtilis* (ATCC 6633), *Enterococcus Faecalis* (ATCC 29212), and *Klebsiella pneumoniae* (ATCC 33152) [9] were obtained from the microbiology department of the University of Lahore. Mueller-Hinton Agar (MHA) was enacted for the growth of bacterial colonies. While the DMSO was used as a preservative for extraction purposes. It was preferred as a negative control and ciprofloxacin as a positive control [10]. Bacterial inoculation was performed in a saline solution (normal pH). Six test tubes were filled with 5ml solution each. One of the methods is Disc Diffusion Assay for testing microorganisms; Muller Hinton Agar (MHA) medium was taken in Petri dishes [11]. The channel paper circles of 5 mm width were set on the agar plates and then loaded with 20µl of plant extract. The plates were placed at a temperature of 37 C for 24 hours. After placing the development restraint zone was measured in millimeters (mm). Another method that was deployed for the detection of microbial activity was the Well diffusion Assay. To assess contamination in Petri dishes, 20 microliters (µl) of Mueller-Hinton Agar (MHA) were discharged into the dishes and left for the growth of microorganisms in an incubator for 24 hours [12]. Plastic straws were used to avoid contamination, first, they were dipped into an ethanol solution and the opening gap between Petri dishes was set at 6mm. Plant extract of 20 µl was taken out in a petri dish, and placed in an incubator at 370 C for 24 hours. The "inhibition zones" (region of zero growth) were measured in millimeters (mm). Phytochemical analysis of *Asparagus officinalis* includes tests for carbohydrates, proteins, alkaloids, saponins and flavonoid compounds. First, the prepared solution was mixed with 2 drops of alcoholic-naphthol solution. Sulfuric acid of 2 ml was added into the prior test tube [13]. The formation of violet rings indicates the presence of carbohydrates. (Molisch's test). Ninhydrin reagent (25%) was added to the extract and heated till boiling. The formation of a Blue-violet color signifies the presence of amino acids or proteins (Ninhydrin test). The filtrate solution was mixed with Wagner's reagent in a test tube. This reagent is prepared by mixing iodine in potassium iodide. The presence of alkaloids is detected by brown or reddish precipitates (Wagner's test). A few drops of sodium hydroxide solution were mixed with the plant extract. An intense yellow color formation was found in the test tube, which becomes colorless, after reacting with dilute acid. This colorless solution confirms flavonoids compounds (Else if the solution does not turn colorless the test result will be negative) [14]. The extract which consists of dry powder is dissolved in distilled water (2ml). Then, it is shaken and allowed to stand for 10 mins. Froth appearance indicates the presence of saponins (Froth test). The test cylinder contained 50 µL of concentrates ranging from 1 to 5 mg/mL. Add 5 mL of 0.1mM DPPH arrangement (4mg/100ml ethanol). It was blended using a vortex mixer and hatched for about 30 minutes at room temperature. It was performed in generally dim spots perused utilizing a spectrophotometer at 517 nm afterwards [15]. An ascorbic corrosive consisting of 10 mg /ml DMSO was used for correlation. The clear was 80% ethanol (v/v). DPPH search

DOI: https://doi.org/10.54393/pbmj.v6i3.854

impact was determined through this technique.

DPPH searching impact (%) = [(AB-AA)/AB] X 100

RESULTS

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed no zones of inhibition with ciprofloxacin at 25 concentrations. The extract (ethanolic) detected antimicrobial activity against both types that is, gram-positive and gram-negative bacteria. Inhibition zones which had a dilution of 25% concentration were recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone against *S. aureus* was 00mm with ciprofloxacin (20mm), *K. pneumoniae* was 00mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 00mm with ciprofloxacin (23mm), *P. aeruginosa* was 00mm with ciprofloxacin (25mm) and *B. subtilis* was 00mm with ciprofloxacin (28mm).

Table 1: Antibacterial properties of *Asparagus* (25mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asparagus	0	28	0
P. aeruginosa	Asp	0	25	0
Enterococcus Faecalis	Asp	0	23	0
K. pneumoniae	Asp	0	21	0
S. aureus	Asp	0	20	0

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed no zones of inhibition with ciprofloxacin at 50% concentration. The extract found antimicrobial activity against both types of bacteria – gram-positive and gram-negative bacteria. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone was 00mm with ciprofloxacin (20mm), *K. pneumoniae* was 00mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 00mm with ciprofloxacin (23mm), *P. aeruginosa* was 00mm with ciprofloxacin (25mm) and *B. subtilis* was 00mm with ciprofloxacin (28mm) against *S. aureus*.

Table 2: Antibacterial properties of *Asparagus* (50mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	0	25	0
P. aeruginosa	Asp	0	25	0
Enterococcus Faecalis	Asp	0	23	0
K. pneumoniae	Asp	0	21	0
S. aureus	Asp	0	20	0

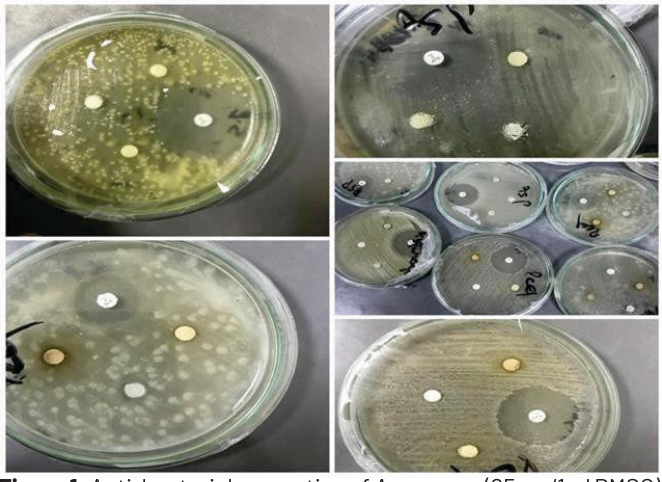

Figure 1: Anti-bacterial properties of *Asparagus* (25mg/1ml DMSO) (50mg/1ml DMSO)

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed maximum zones of inhibition with ciprofloxacin at 75% concentration. Antimicrobial activity was found in the extract against both types, i.e., gram-positive and gram-negative bacteria. An inhibition zone of dilution of 75% concentration was recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The result showed that the inhibition zone was 30mm with ciprofloxacin (20mm), *K. pneumoniae* was 13mm with ciprofloxacin (21mm), *Enterococcus Faecalis* was 12mm with ciprofloxacin (23mm), *P. aeruginosa* was 9mm with ciprofloxacin (25mm) and *B. subtilis* was 10mm with ciprofloxacin (28mm) against *S. aureus*.

Table 3: Antibacterial property of *Asparagus* (75mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	10	25	0
P. aeruginosa	Asp	9	25	0
Enterococcus Faecalis	Asp	12	23	0
K. pneumoniae	Asp	13	21	0
S. aureus	Asp	30	20	0

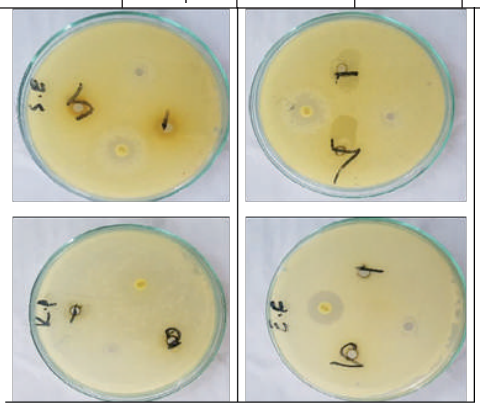

Figure 2: Antibacterial property of *Asparagus* plant (75mg/1ml

DOI: https://doi.org/10.54393/pbmj.v6i3.854

DMSO)

Try to adjust this on the same page

The ethanolic extract used against *S. aureus*, *K. pneumoniae*, *Enterococcus Faecalis*, *P. aeruginosa* and *B. subtilis* showed maximum zones of inhibition with ciprofloxacin at 100% concentration. The extract (ethanolic) detected antimicrobial activity as opposed to both types, that are, gram-positive and gram-negative bacteria. An inhibition zone of dilution of 100% concentration was recorded against bacterial strains. To make dilutions, the ethanolic extract was dissolved in 1ml DMSO which was also used as a negative control in pure form and showed no zone of inhibition. The results showed that the inhibition zone was 40mm with ciprofloxacin (20mm), *K. pneumoniae* was 26mm with ciprofloxacin (21mm), *Enterococcus faecalis* was 29mm with ciprofloxacin (23mm), *P. aeruginosa* was 11mm with ciprofloxacin (25mm) and *B. subtilis* was 14 mm with ciprofloxacin(28mm)against *S. aureus*.

Table 4: Antibacterial property of *Asparagus*(100mg/1ml DMSO)

Bacteria	Plant part	Zone of inhibition	Positive control	Negative control
B. subtilis	Asp	14	20	0
P. aeruginosa	Asp	11	25	0
K. pneumoniae	Asp	29	23	0
Enterococcus Faecalis	Asp	26	21	0
S. aureus	Asp	40	20	0

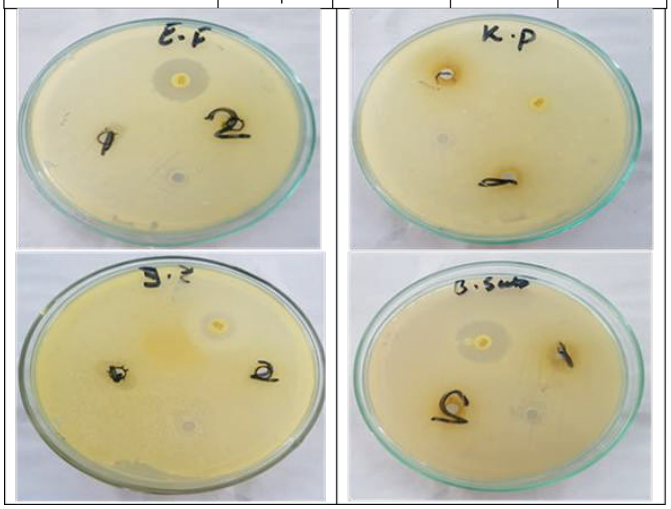

Figure 3: Antibacterial properties of *Asparagus* plant (100mg/1ml DMSO)

Table 5: Results of phytochemical analysis of *Asparagus officinalis*

Phytochemicals	Tests	Result
Carbohydrates	Molisch	Positive
Proteins	Ninhydrin	Negative
Alkaloids	Wagner	Negative
Saponins	Froth	Positive
Proteins	Biuret	Negative
Phenols	Ferric chloride	Positive

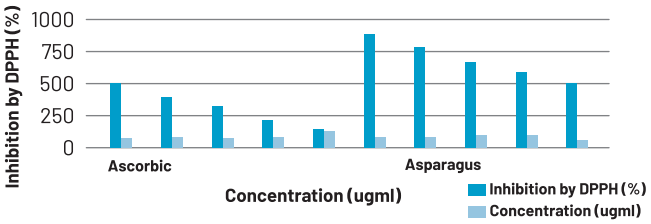

Figure 4: Graph showing antioxidant activity of *Asparagus* ethanolic extract by comparing with ascorbic acid DPPH inhibition

DISCUSSION

The proposed study was based on the medicinal activities of *Asparagus officinalis*. Antibacterial activity of *Asparagus* plant extract demonstrated zones of inhibition against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 33152). A group of study conducted by Amare in 2015 they found that *S. aureus* exhibited larger zones of inhibition when treated with ethanolic extracts of the *Asparagus* plant. Our results are in line with the previously work done [18]. In a study presented by Sriyab *et al.*, observed that only the alcoholic extract of turnip, rather than the extract of *Brassica napus L.*, exhibited zones of inhibition against *Pseudomonas aeruginosa* [19]. Inhibition zones of 2, 8, and 6 mm were observed on three consecutive days, respectively. There was no significant effect in the well diffusion assay. In comparison, this study found that a 25 mg/1 ml DMSO solution produced fewer zones of inhibition than a 50 mg solution. *Proteus* exhibited larger zones of inhibition in the 25 mg solution than in the 50 mg solution, while *P. aeruginosa* showed more zones of inhibition in the 50 mg solution than in the 25 mg solution. A similar study was elaborated by Olivier *et al* and showed that *S. aureus* was more sensitive to the plant extract [20]. Our results showed the potent activity of *Asparagus officinalis* as the plant was enriched with phytochemicals. Similar results were presented in 2019 by a group of scientists who elaborated the phytochemical contents [21]. Our results in line with another study which was conducted by Linka *et al.*, and Jianu *et al.*, they screened vitamin C, phytic acid, fiber content and tocopherol [22, 23]. Similar results were presented by Pandey in 2013 they confirmed the potent antioxidant activity of the plant [24]. No experiment was conducted on ethanolic leaves extract of *Asparagus officinalis* that depicts the novelty of the proposed study. For human consumption, Asparagus has been an admirable vegetable since its early domestication, especially by virtue of its medicinal properties. Nowadays, its cultivation has been transmitted across the globe in all continents including Europe, Africa and beyond. Considering the escalating demand of consumers for

DOI: https://doi.org/10.54393/pbmj.v6i3.854

fresh, frozen and canned asparagus more and more area has been acquired for its cultivation. In recent study work, different experiments have been performed to identify and characterize the specific phytochemicals. By concluding all of the above research, it has been confirmed that Asparagus leaves have excellent antimicrobial activity. The business community has also shown interest in manufacturing new drugs for the therapy of distinct diseases in both research institutes and pharmaceutical industries. Leaves extract is a promising candidate for the use of human health as an antioxidant based on natural products. Lastly, it is suggested that there are many emerging possibilities and strategies that can allow the effective growth of Asparagus leaf system extracts to be applied as a natural element of food preservation, cosmetics and medicinal products such as they have strong antioxidant, antimicrobial and phytochemical effects.

CONCLUSIONS

By concluding all of the above research, it has been confirmed that Asparagus leaves have excellent antimicrobial activity. The business community has also shown interest in manufacturing new drugs for the therapy of distinct diseases in both research institutes and pharmaceutical industries. Leaves extract is a promising candidate for the use of human health as an antioxidant based on natural products. Lastly, it is suggested that there are many emerging possibilities and strategies that can allow the effective growth of Asparagus leaf system extracts to be applied as a natural element of food preservation, cosmetics and medicinal products such as they have strong antioxidant, antimicrobial and phytochemical effects.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Pegiou E, Mumm R, Acharya P, de Vos RC, Hall RD. Green and white asparagus (Asparagus officinalis): A source of developmental, chemical and urinary intrigue. Metabolites. 2019 Dec 25; 10(1): 17. doi: 10.3390/metabo10010017

[2] Soteriou GA, Antoniou C, Rouphael Y, Kyratzis AC, Kyriacou MC. Changes in the primary and secondary metabolome of male green asparagus (Asparagus officinalis L.) as modulated by sequential harvesting. Food Chemistry. 2021 Oct; 358: 129877. Doi: 10.1016/j.foodchem.2021.129877

[3] Alyami NM, Almeer R, Alyami HM. Protective effects of Asparagus officinalis (asparagus) against lead toxicity in mice. Environmental Science and Pollution Research. 2023 Feb; 30(7): 18718-30. doi: 10.1007/s11356-022-23540-5

[4] Yang C, Ye Z, Mao L, Zhang L, Zhang J, Ding W, *et al*. Analysis of volatile organic compounds and metabolites of three cultivars of asparagus (Asparagus officinalis L.) using E-nose, GC-IMS, and LC-MS/MS. Bioengineered. 2022 Apr; 13(4): 8866-80. doi: 10.1080/21655979.2022.2056318

[5] Olivier MT, Muganza FM, Shai LJ, Gololo SS, Nemutavhanani LD. Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of Asparagus suaveolens aerial parts. South African Journal of Botany. 2017 Jan; 108: 41-6. doi: 10.1016/j.sajb.2016.09.014

[6] Desoukey SF, El-Nahas SE, Sabh AZ, Taha ZK, El-Shabrawi HM. Antimicrobial effect of Asparagus officinalis L. extracts. Plant Archives. 2020; 20(2): 9253-64.

[7] Zhang H, Birch J, Xie C, Yang H, Bekhit AE. Optimization of ultrasound assisted extraction method for phytochemical compounds and in-vitro antioxidant activity of New Zealand and China Asparagus cultivars (officinalis L.) roots extracts. Food chemistry. 2019 Oct; 294: 276-84. doi: 10.1016/j.foodchem.2019.03.012

[8] Hamdi A, Jaramillo-Carmona S, Rodríguez-Arcos R, Jiménez-Araujo A, Lachaal M, Karray-Bouraoui N, *et al*. Phytochemical Characterization and Bioactivity of Asparagus acutifolius: A Focus on Antioxidant, Cytotoxic, Lipase Inhibitory and Antimicrobial Activities. Molecules. 2021 Jun; 26(11): 3328. doi: 10.3390/molecules26113328

[9] Begum A, Sindhu K, Giri K, Umera F, Gauthami G, Kumar JV, *et al*. Pharmacognostical and physio-chemical evaluation of Indian Asparagus officinalis Linn family Lamiaceae. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9: 327-36.

[10] Karim M, Boikess RS, Schwartz RA, Cohen PJ. Dimethyl sulfoxide (DMSO): a solvent that may solve selected cutaneous clinical challenges. Archives of Dermatological Research. 2022 Dec: 1-8. doi: 10.1007/s00403-022-02494-1

[11] Khalili H, Soltani R, Negahban S, Abdollahi A, Gholami K. Reliability of disk diffusion test results for the antimicrobial susceptibility testing of nosocomial gram-positive microorganisms: is E-test method better?. Iranian Journal of Pharmaceutical Research: IJPR. 2012; 11(2): 559.

DOI: https://doi.org/10.54393/pbmj.v6i3.854

[12] Ngamsurach P and Praipipat P. Comparative antibacterial activities of Garcinia cowa and Piper sarmentosum extracts against Staphylococcus aureus and Escherichia coli with studying on disc diffusion assay, material characterizations, and batch experiments. Heliyon. 2022 Nov; 8(11): e11704. doi: 10.1016/j.heliyon.2022.e11704

[13] Jamal Uddin AF, Taufique T, Mayda U, Roni MZ, Mehraj H. Yield performance and phytochemical screening of three asparagus varieties. Bangladesh Research Publication Journal. 2014; 10(2): 196-201.

[14] Zhang H, Birch J, Pei J, Mohamed Ahmed IA, Yang H, Dias G, *et al*. Identification of six phytochemical compounds from Asparagus officinalis L. root cultivars from New Zealand and China using UAE-SPE-UPLC-MS/MS: effects of extracts on H2O2-induced oxidative stress. Nutrients. 2019 Jan; 11(1): 107. doi: 10.3390/nu11010107

[15] Fathalipour M, Delnavazi MR, Safa O, Zarifinia N, Rafiee B. Antioxidant and antinociceptive effects of hydroalcoholic root extract of Asparagus officinalis L. Physiology and Pharmacology. 2020 Dec; 24(4): 322-30. doi: 10.32598/ppj.24.4.30

[16] Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, *et al*. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in microbiology. 2018 Jul; 9: 1639. doi: 10.3389/fmicb.2018.01639

[17] Battu GR and Kumar BM. Phytochemical and antimicrobial activity of leaf extract of Asparagus racemosus Willd. Pharmacognosy Journal. 2010 Aug; 2(12): 456-63. doi: 10.1016/S0975-3575(10)80031-8

[18] Amare A, Hadush A, Aregawi H, Kide N. Antibacterial activity of oil extracts of Black Mustard (Brassica nigra) seeds against bacteria isolated from fresh juice in selected areas of Axum town. International Journal of Innovations in Science & Technology. 2015; 4(4): 15-8.

[19] Sriyab S, Laosirisathian N, Punyoyai C, Anuchapreeda S, Tima S, Chiampanichayakul S, Chaiyana W. Nutricosmetic effects of Asparagus officinalis: A potent matrix metalloproteinase-1 inhibitor. Scientific Reports. 2021 Apr; 11(1): 1-1. doi: 10.1038/s41598-021-88340-2

[20] Olivier MT, Muganza FM, Shai LJ, Gololo SS, Nemutavhanani LD. Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of Asparagus suaveolens aerial parts. South African Journal of Botany. 2017 Jan; 108: 41-6. doi: 10.1016/j.sajb.2016.09.014

[21] Mashele SS. Medicinal properties of selected asparagus species: a review. Phytochemicals in Human Health. 2019 Aug.

[22] Linka WA, Wojtaszek I, Zgoda MM, Kołodziejczyk MK. The Use of Synthetic Polymers (Superdisintegrants) in Technology Tablets Containing Ethanol Dry Extract from Asparagus officinalis. Polymers in Medicine. 2015; 45(2): 73-80. doi: 10.17219/pim/61167

[23] Jianu C, Moleriu R, Stoin D, Cocan I, Bujancă G, Pop G, *et al*. Antioxidant and antibacterial activity of Nepeta× faassenii bergmans ex stearn essential oil. Applied Sciences. 2021 Jan; 11(1): 442. doi: 10.3390/app11010442

[24] Pandey M, Mohd Amin MC, Ahmad N, Abeer MM. Rapid synthesis of superabsorbent smart-swelling bacterial cellulose/acrylamide-based hydrogels for drug delivery. International Journal of Polymer Science. 2013 Jan; 2013. doi: 10.1155/2013/905471

Take reference 21 on the next page

DOI: https://doi.org/10.54393/pbmj.v6i3.852

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 3 (March 2023)

Original Article

Chronic Pulmonary Obstructive Disease (COPD) On High Resolution Computed Tomography

Shahraz Ashraf[1*], Ali Junaid Dar[1] and Urwa Nasir[1]

[1]University Institute of Radiological Sciences and Medical Imaging Technology, The University of Lahore, Gujrat, Pakistan

ARTICLE INFO

Key Words:

COPD, HRCT, Walk Test, FEVI, FVC

How to Cite:

Ashraf, S. ., Junaid Dar, A. ., & Nasir, U. . (2023). Chronic Pulmonary Obstructive Disease (COPD) On High Resolution Computed Tomography: COPD on High Resolution Computed Tomography. Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.852

***Corresponding Author:**

Shahraz Ashraf

University Institute of Radiological Sciences and Medical Imaging Technology, The University of Lahore, Gujrat, Pakistan

shahrazashraf809@gmail.com

Received Date: 5th March, 2023

Acceptance Date: 27th March, 2023

Published Date: 31st March, 2023

ABSTRACT

The prevalent, preventable, and treatable chronic lung illness known as chronic obstructive pulmonary disease (COPD), which may be accurately detected on HRCT, affects both men and women worldwide. **Objective:** To evaluate the diagnostic features of chronic pulmonary obstructive disease (COPD) using high resolution computed tomography. **Methods:** This study included 120 patients with COPD at least having a comprehensive clinical record of 6MWT defined as COPD by a post-bronchodilator FEV1/FVC 70% with sustained expiratory flow limitation. The sample size was computed at 120 patients using convenient approach and non-contrast HRCT was performed using 64 slides scanning from the apex of the lung to the diaphragm. Emphysema scoring and -950 HU criteria were used to automatically partition the lungs without including the central airways. The data were entered and analyzed on SPSS version 22. **Results:** HRCT scan findings show that patients with parenchymal bands were 9(7.5%) with bronchial wall thickening, nodules were (24)20%, bronchiectasis were (23)19%, apical fibrosis were (19)15%, and tree on bud pattern were (12)10%. **Conclusions:** It is concluded that COPD is common in males and worsens in cigarette or tobacco smokers, with a prevalence of parenchymal bands, bronchial wall thickening, nodules, bronchiectasis, apical fibrosis, and tree-on-bud patterns.

INTRODUCTION

Smoking has been established as the primary cause of chronic obstructive pulmonary disease (COPD), which is increasingly becoming a global public health problem [1]. Chronic inflammatory pulmonary disease, usually known COPD, decreases lung airflow [2]. The hallmark of COPD is tissue alterations that cause the walls of the airways to thicken and become blocked [3]. As the illness progresses, there is an increase in mucosal metaplasia, sub mucosal hypertrophy, per bronchial fibrosis, and airway smooth muscle mass [4]. Worldwide, men and women are both affected by the widespread, preventable, and curable chronic lung condition known as COPD [5]. For the past 20 years, smoking has been the main contributor of COPD in both industrialized and developing countries [6]. One's likelihood of developing COPD increases with the amount of cigarettes they smoke [7]. However, some smokers can quit for years without developing COPD [8]. In 2019, 3.23 million people worldwide died from chronic pulmonary obstructive disease, which is the third highest cause of mortality globally [9]. A low or middle-income nation accounts for 90% of COPD deaths among people under the age of 70. According to recent research on the topic, 50% of smokers eventually develop COPD, which is described by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [10, 11]. Another research that divided patients into emphysema and airway-predominant groups revealed that emphysema patients had lower FEV1, greater functional limitations; higher BODE scores, and lower BMI. They were also more severely affected by the disease. Four groups of participants with distinct characteristics were

DOI: https://doi.org/10.54393/pbmj.v6i3.852

effectively created by the researcher. It is possible to measure the numerous morphological characteristics of lung illnesses using HRCT pictures and abnormalities [12]. The patient's clinical history, symptoms, including chronic severe dyspnea, and pulmonary function tests, which showed irreversible airflow limitation, was all used to determine the existence of COPD in accordance with the GOLD criteria [13]. The mortality rate of COPD is steadily increasing, making it the fourth leading cause of death worldwide [14]. The leading disease and mortality cause in the world is COPD, which has a substantial and growing economic and social impact [15]. The lungs of a person with chronic obstructive pulmonary disease become worse over time [16]. Smoking has been recognized as the main risk factor for COPD, along with chronic bronchitis and emphysema, which are further symptoms of the illness [17, 18]. The purpose of this study was to examine the relationships between symptoms, lung function, physical changes that are measured and have clinical significance and the degree of emphysema assessed using HRCT in COPD patients, as well as the relationship between smoking and COPD [19, 20]. It is now possible to advise smokers that if they continue to smoke throughout their lives, they have at least a one in two probability of acquiring COPD [21]. The results of this study will help radiologists identify COPD on HRCT patients.

METHODS

It was descriptive cross-sectional research at the teaching hospital Aziz Bhatti Shaheed Teaching Hospital (Gujrat Punjab Pakistan). Patients who have completed a 6-minute walking test (6MWT) in the respiratory therapy division were included. Younger people under the age of 40 were excluded. Based on the correlations of the HRCT results, the sample size was computed at 120 patients. A non-contrast High Resolution Computed Tomography was performed from the apex of the lung to the diaphragm. The Medical Research Council (MRC) questionnaire, which is commonly used to grade the influence of dyspnea on everyday activities, was used to assess dyspnoea. It has been linked to various indicators of health status and prognosis. The MRC scale runs from not bothered by breathlessness unless during severe activity to breathless when dressing or undressing. To assess symptoms and functional status, the clinical COPD questionnaire (CCQ) was employed. It has been verified for clinical control trials in COPD patients. The 10 item CCQ is self-administered, and patients are asked to recollect symptoms from the preceding week. Each item is rated on a scale of 0 to 6, and the overall CCQ score is computed by dividing the whole sum by the number of things. Hence, CCQ spans from 0 (excellent control) to 6 (Extremely poor control). A body plethysmograph (Master Screen Body/Diffusion; Viasys Healthcare) was used to evaluate FEV1, vital capacity (VC), total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), and carbon monoxide diffusion capacity (DLCO). Spirometry was done in accordance with Swedish Board for Accreditation and Conformity Assessment (SWEDAC) accreditation, meeting the norms of ISO/IEC 17025. All measured values were represented as a percentage of expected (e.g., % FEV1). The absolute ratio of FEV1/VC was also shown. The value of VC provides the best of both forced VC (FVC) and gradual VC. HRCT scanning was conducted with the patients in the supine position, using a multi detector CT scanner, to cover the whole lung. The lung algorithm was used to build 1 mm thick trans axial images; patients were assessed with HRCT. HRCT scans were visually evaluated with a focus on the type, location, and amount of emphysema. The degree of emphysema was calculated as a percentage of total lung volume (Emphysema HRCT). Findings included bronchiectasis, bronchial wall thickening, and mucus plugs, which were not further investigated in this study. The review was conducted by an experienced chest radiologist who was not aware of the V/P SPECT data. V/P SPECT The V/P SPECT was done in accordance with the European Association of Nuclear Medicine's (EANM) recommendations. In short, a broad field of view dual-head gamma camera with a low-energy collimator was employed. The acquisition was done in a 64 64 matrix, magnified to a pixel size of 6.8 mm, using 128 projections across 360°. During the breathing research, 64 steps of 10 seconds each were employed, and for the perfusion study, 5 seconds were used. The whole acquisition time was around 20 minutes, and all patients tolerated it well. V/P SPECT was done on a single day. The test began with the inhalation of Techne gas (Cyclomedica Ltd.) until 30 MBq had been delivered to the lungs. After that, ventilation tomography was performed. Following that, 100-120 MBq of 99mTc-labeled human albumin macroaggregates (Malinckrodt Medical BV) were progressively given intravenously without patient movement and in a carefully maintained supine posture. The procedure was then followed by perfusion tomography. With this procedure, the effective dosage is 1.8 mSv. V/P SPECT images were provided for blinded assessment by an independent technician after reconstruction.

RESULTS

Table 1 represents the data about the age having 3-subgroups, 60-69 years age group were mostly affected by COPD 70(58.3%), 50-59 were affected 45(37.5%) and only 5(4.2%) were affected during the age group of 40-49. The prevalence percentage of chronic obstructive pulmonary

DOI: https://doi.org/10.54393/pbmj.v6i3.852

disease was observed more in males 67(55.8%) and 53(44.2%) in females only.

Table 1: Age, gender, and smoking effect

Variables		Frequency (%)
Age	40-49	5(4.2)
	50-59	45(37.5)
	60-69	70(58.3)
	Total	120 (100)
Gender	Male	67(55.8)
	Female	53(44.2)
	Total	120(100)
Smoking	Smoker	99(82.5)
	nonsmoker	21(17.5)
	Total	120(100)

Table 2 represents that the exposure to the toxic gases such as the wood smoke factory worker. 72(60%) patients were affected from the exposure to toxic gases and 48(40%) were never experienced any toxic gas. The smokers have more risks to getting the chronic pulmonary obstructive disease. 99(82.5%) patients were smokers and only 21(17.5%) were non-smokers. Exposure to the toxic gases such as the wood smoke factory worker. 72(60%) patients were affected from the exposure to toxic gases and 48(40%) were never experienced any toxic gas. The HRCT scan findings 5(4.3%) COPD patients have the chronic obstructive pulmonary disease with normal parenchymal bands, COPD patients with parenchymal bands were 9(7.5%), with bronchial wall thickening were 28(23.3%), nodules were 24(20%) bronchiectasis were 23(19%), apical fibrosis were 19(15%) and the most chronic from that is tree on bud pattern are 12(10 %) in the chronic obstructive pulmonary disease.

Table 2: Clinical history and HRCT findings

Variables		Frequency (%)
Exposure to the toxic gases	Yes	72(60.0)
	No	48(40.0)
	Total	120 (100)
	Normal	5(4.2)
HRCT finding in the COPD	Parenchymal bands	9(7.5)
	Bronchial wall thickening	28(23.3)
	Nodules	24(20)
	Bronchiectasis	23(19.2)
	Apical fibrosis	19(15.8)
	Tree in bud pattern	12(10)
	Total	120(100)

Figure 1A shows hyper inflated lung with flattening of right hemi diaphragm. Figure 1B shows herniation of right upper lobe towards left. Figure 1C shows thin-walled cysts early changes of COPDs. Figure 1D shows thick-walled cysts are noted that cause emphysematous changes.

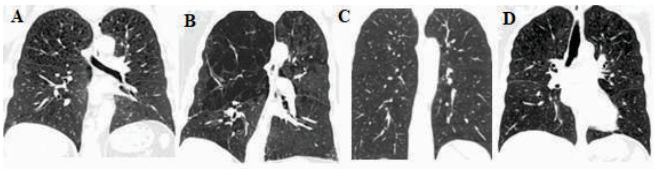

Figure 1: High resolution computed tomographic (HRCT) images

Table 3 shows that the chronic pulmonary obstructive disease patients have low capacity of exercise in the above COPD patients have a test of 6 mint walk about the 78(65%) patients get tired during the walk text and 42(35%) were normal after the six min walk test. 95(79.2%) patients of the chronic obstructive pulmonary disease were obese, and 25(20.8%) were normal. Obesity was one of the major causes of the pulmonary restrictive disease.

Table 3: BMI and Exercise Capacity

Variables		Frequency (%)
Exercise capacity (6 mints walk test)	Normal	42(35)
	Abnormal	78(65)
	Total	120(100)
Body Mass index	Normal	25(20.8)
	Obese	95(79.2)
	Total	120(100)

DISCUSSION

With a global incidence of 10.1% in adults 40 years of age or older, COPD is a prevalent, preventable, and curable condition [22]. COPD, a condition that is rapidly impacting public health across the world, has been linked to smoking as its main cause [23]. The more cigarettes someone smokes, the more likely they are to get COPD. Some smokers, meanwhile, can stop for years without acquiring COPD. In the current study, data were collected from 120 patients and non-contrast HRCT was performed. Kesimer *et al.*, discovered that men account for 76% of COPD prevalence, which is consistent with our study's findings that men make up the majority of the afflicted patients 67(55.8%) men with COPD [24]. Another research found that the prevalence of chronic obstructive pulmonary disease (COPD) was 9.23% in men and 6.16% in women throughout the population, indicating that it is no more a condition that just affects males [25]. Women may be more sensitive to the effects of cigarette smoke due to the higher prevalence of COPD in women, although smoking less than males [26]. Kojima *et al.*, unlike our study, it was discovered that the age group (25-49) has the highest prevalence of COPD in Japan. According to our study most affected age group was (60-69) [27]. Contrarily, a large number of other studies find that the prevalence of COPD increased dramatically with ageing, from 1.9% in the 40–49 year age group to 28.6% in the group of those over 70 years [28]. While some studies suggest that adults over 60 have a two- to three-fold greater frequency of COPD than do people in younger age groups [29]. The results of the

DOI: https://doi.org/10.54393/pbmj.v6i3.852

current study were similar to the results of Moreira *et al.*, which states that patients with wood smoke-related COPD had lung abnormalities shown on HRCT scans that were much more severe than those in the control group, who had no history of exposure to wood smoke. Another finding that was similar to our study was that the COPD group had considerably more parenchymal bands, thickened bronchial walls, bronchiectasis, mosaic perfusion patterns, and laminar atelectasis than did the control group [30].

CONCLUSIONS

COPD can be developed in any age group and common in males but worsens in cigarette or tobacco smokers. The condition progresses, thickening the bronchial wall causing emphysema. There were 20% nodules, 19% bronchiectasis cases, 15% apical fibrosis cases, and 10% of COPD cases had a tree-on-bud pattern. High Resolution Computed Tomography (HRCT) play important role in the diagnosis of chronic obstructive pulmonary disease. HRCT is golden modalities in lung interstitial disease, such as tuberculosis, bronchitis, pneumonia, and chronic obstructive pulmonary disease. With attention to reduce dose with concern to ALARA principle.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Laniado-Laborín R. Smoking and chronic obstructive pulmonary disease (COPD). Parallel epidemics of the 21st century. International Journal of Environmental Research and Public Health. 2009 Jan; 6(1): 209-24. doi: 10.3390/ijerph6010209

[2] Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. The Lancet. 2004 Aug; 364(9435): 709-21. doi: 10.1016/S0140-6736(04)16900-6

[3] Aoshiba K and Nagai A. Differences in airway remodeling between asthma and chronic obstructive pulmonary disease. Clinical Reviews in Allergy & Immunology. 2004 Aug; 27: 35-43. doi: 10.1385/CRIAI:27:1:035

[4] Jeffery PK. Structural and inflammatory changes in COPD: a comparison with asthma. Thorax. 1998 Feb; 53(2): 129. doi: 10.1136/thx.53.2.129

[5] Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, *et al.* Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet Respiratory Medicine. 2017 Sep; 5(9): 691-706.

[6] Boschetto P, Quintavalle S, Miotto D, Lo Cascio N, Zeni E, Mapp CE. Chronic obstructive pulmonary disease (COPD) and occupational exposures. Journal of Occupational Medicine and Toxicology. 2006 Dec; 1: 1-6. doi: 10.1186/1745-6673-1-11

[7] Petrescu F, Voican SC, Silosi I. Tumor necrosis factor-α serum levels in healthy smokers and nonsmokers. International Journal of Chronic Obstructive Pulmonary Disease. 2010 Aug: 217-22. doi: 10.2147/COPD.S8330

[8] Eklund BM, Nilsson S, Hedman L, Lindberg I. Why do smokers diagnosed with COPD not quit smoking?-a qualitative study. Tobacco Induced Diseases. 2012 Dec; 10(1): 1-7. doi: 10.1186/1617-9625-10-17

[9] Yang X, Zhang T, Zhang Y, Chen H, Sang S. Global burden of COPD attributable to ambient PM2. 5 in 204 countries and territories, 1990 to 2019: A systematic analysis for the Global Burden of Disease Study 2019. Science of the Total Environment. 2021 Nov; 796: 148819. doi: 10.1016/j.scitotenv.2021.148819

[10] Siddharthan T, Wosu AC, Pollard SL, Hossen S, Alupo P, Shade T, *et al.* A novel case-finding instrument for chronic obstructive pulmonary disease in low-and middle-income country settings. International Journal of Chronic Obstructive Pulmonary Disease. 2020 Nov: 2769-77. doi: 10.2147/COPD.S268076

[11] Bozkus F, Dikmen N, Samur A. Microalbuminuria in subjects with COPD: relationship to the new version of global initiative for chronic obstructive lung disease staging. Respiratory Care. 2017 Mar; 62(3): 307-14. doi: 10.4187/respcare.05168

[12] Bartholmai BJ, Raghunath S, Karwoski RA, Moua T, Rajagopalan S, Maldonado F, *et al.* Quantitative CT imaging of interstitial lung diseases. Journal of Thoracic Imaging. 2013 Sep; 28(5). doi: 10.1097/RTI.0b013e3182a21969

[13] Kitaguchi Y, Komatsu Y, Fujimoto K, Hanaoka M, Kubo K. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. International Journal of Chronic Obstructive Pulmonary Disease. 2012 Apr: 283-9. doi: 10.2147/COPD.S30651

[14] Adeloye D, Basquill C, Papana A, Chan KY, Rudan I, Campbell H. An estimate of the prevalence of COPD in Africa: a systematic analysis. COPD: Journal of Chronic Obstructive Pulmonary Disease. 2015 Jan; 12(1): 71-81. doi: 10.3109/15412555.2014.908834

[15] López-Campos JL, Tan W, Soriano JB. Global burden

DOI: https://doi.org/10.54393/pbmj.v6i3.852

of COPD. Respirology. 2016 Jan; 21(1): 14-23. doi: 10.1111/resp.12660

[16] Lohne V, Heer HC, Andersen M, Miaskowski C, Kongerud J, Rustøen T. Qualitative study of pain of patients with chronic obstructive pulmonary disease. Heart & Lung. 2010 May; 39(3): 226-34. doi: 10.1016/j.hrtlng.2009.08.002

[17] Brandsma CA, Van den Berge M, Hackett TL, Brusselle G, Timens W. Recent advances in chronic obstructive pulmonary disease pathogenesis: from disease mechanisms to precision medicine. The Journal of Pathology. 2020 Apr; 250(5): 624-35. doi: 10.1002/path.5364

[18] Salvi S. Tobacco smoking and environmental risk factors for chronic obstructive pulmonary disease. Clinics in Chest Medicine. 2014 Mar; 35(1): 17-27. doi: 10.1016/j.ccm.2013.09.011

[19] Jögi J, Ekberg M, Jonson B, Bozovic G, Bajc M. Ventilation/perfusion SPECT in chronic obstructive pulmonary disease: an evaluation by reference to symptoms, spirometric lung function and emphysema, as assessed with HRCT. European Journal of Nuclear Medicine and Molecular Imaging. 2011 Jul; 38: 1344-52. doi: 10.1007/s00259-011-1757-5

[20] Grydeland TB, Dirksen A, Coxson HO, Eagan TM, Thorsen E, Pillai SG, *et al*. Quantitative computed tomography measures of emphysema and airway wall thickness are related to respiratory symptoms. American Journal of Respiratory and Critical Care Medicine. 2010 Feb; 181(4): 353-9. doi: 10.1164/rccm.200907-1008OC

[21] Politis A, Ioannidis V, Gourgoulianis KI, Daniil Z, Hatzoglou C. Effects of varenicline therapy in combination with advanced behavioral support on smoking cessation and quality of life in inpatients with acute exacerbation of COPD, bronchial asthma, or community-acquired pneumonia: A prospective, open-label, preference-based, 52-week, follow-up trial. Chronic Respiratory Disease. 2018 May; 15(2): 146-56. doi: 10.1177/1479972317740128

[22] Diaz-Guzman E and Mannino DM. Epidemiology and prevalence of chronic obstructive pulmonary disease. Clinics in Chest Medicine. 2014 Mar; 35(1): 7-16. doi: 10.1016/j.ccm.2013.10.002

[23] Anto JM, Vermeire P, Vestbo J, Sunyer J. Epidemiology of chronic obstructive pulmonary disease. European Respiratory Journal. 2001 May; 17(5): 982-94. doi: 10.1183/09031936.01.17509820

[24] Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, *et al*. Airway mucin concentration as a marker of chronic bronchitis. New England Journal of Medicine. 2017 Sep; 377(10): 911-22. doi: 10.1056/NEJMoa1701632

[25] Ntritsos G, Franek J, Belbasis L, Christou MA, Markozannes G, Altman P, *et al*. Gender-specific estimates of COPD prevalence: a systematic review and meta-analysis. International Journal of Chronic Obstructive Pulmonary Disease. 2018 May: 1507-14. doi: 10.2147/COPD.S146390

[26] Barnes PJ. Sex differences in chronic obstructive pulmonary disease mechanisms. American journal of respiratory and critical care medicine. 2016 Apr; 193(8):813-4. doi: 10.2147/COPD.S146390

[27] Kojima S, Sakakibara H, Motani S, Hirose K, Mizuno F, Ochiai M, *et al*. Incidence of chronic obstructive pulmonary disease, and the relationship between age and smoking in a Japanese population. Journal of epidemiology. 2007; 17(2): 54-60. doi: 10.2188/jea.17.54

[28] Chen J, Yin Y, Zhang Y, Lin X, Chen T, Yang Z, *et al*. Chronic Obstructive Pulmonary Disease Prevalence and Associated Risk Factors in Adults Aged 40 Years and Older in Southeast China: A Cross-Sectional Study During 2019–2020. International Journal of Chronic Obstructive Pulmonary Disease. 2022 Jan: 2317-28. doi: 10.2147/COPD.S377857

[29] Gutiérrez F, Masiá M, Mirete C, Soldán B, Rodríguez JC, Padilla S, *et al*. The influence of age and gender on the population-based incidence of community-acquired pneumonia caused by different microbial pathogens. Journal of infection. 2006 Sep; 53(3): 166-74. doi: 10.1016/j.jinf.2005.11.006

[30] Moreira MA, Barbosa MA, Queiroz MC, Teixeira KI, Santana Júnior PJ, Montadon Júnior ME, Jardim JR. Pulmonary changes on HRCT scans in nonsmoking females with COPD due to wood smoke exposure. Jornal Brasileiro de pneumologia. 2013 Mar; 39: 155-63. doi: 10.1590/S1806-37132013000200006

DOI: https://doi.org/10.54393/pbmj.v6i3.853

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
Volume 6, Issue 3 (March 2023)

Original Article

Computational Prediction of *Nigella sativa* Compounds as Potential Drug Agents for Targeting Spike Protein of SARS-CoV-2

Laraib Ali[1], Rashid Saif [2,3*], Muhammad Hassan Raza[2], Muhammad Osama Zafar[2], Saeeda Zia[4], Mehwish Shafiq[1], Tuba Ahmad[5] and Iram Anjum[1]

[1]Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan
[2]Decode Genomics, Punjab University Employees Housing Scheme, Lahore, Pakistan
[3]Department of Biotechnology, Qarshi University, Lahore, Pakistan
[4]Department of Sciences and Humanities, National University of Computer and Emerging Sciences, Lahore, Pakistan
[5]Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan

ARTICLE INFO

Key Words:
SARS-CoV-2, Spike Protein, COVID-19, MOE, Molecular Docking, MDS Analysis
How to Cite:
Ali, L. ., Saif, R., Hassan Raza, M. ., Osama Zafar, M. ., Zia, S. ., Shafiq, M. ., Ahmad, T. ., & Anjum, I. . (2023). Computational Prediction of Nigella sativa Compounds as Potential Drug Agents for Targeting Spike Protein of SARS-CoV-2: Computational Prediction of N. sativa Compounds as Potential Drug Agents . Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.853
***Corresponding Author:**
Rashid Saif
Decode Genomics,Punjab University Employees Housing Scheme, Lahore, Pakistan
rashid.saif37@gmail.com
Received Date: 14th February, 2023
Acceptance Date: 25th March, 2023
Published Date: 31st March, 2023

ABSTRACT

SARS-CoV-2 was first identified in Wuhan, China in December 2019 and has rapidly devastated worldwide. The lack of approved therapeutic drugs has intensified the global situation, so researchers are seeking potential treatments using regular drug agents and traditional herbs as well. **Objectives:** To identify new therapeutic agents from *Nigella sativa* against spike protein (PDB ID: 7BZ5) of SARS-CoV-2. **Methods:** The 46 compounds from *N. sativa* were docked with spike protein using Molecular Operating Environment (MOE) software and compared with commercially available anti-viral drugs e.g., Arbidol, Favipiravir, Remdesivir, Nelfinavir, Chloroquine, Hydroxychloroquine. The Molecular Dynamic Simulation (MDS) analysis was also applied to determine ligand-protein complex stability. Furthermore, the pharmacological properties of compounds were also analyzed using AdmetSAR and SwissADME. **Results:** Out of its total 46 ligands, 8 compounds i.e., Methyl stearate, Eicosadienoic acid, Oleic acid, Stearic acid, Linoleic acid, Myristoleic acid, Palmitic acid, and Farnesol were selected for further analysis based on their minimum binding energy ranges from -7.45 to -7.07 kcal/mol. The docking scores of *N. sativa* phytocompounds were similar to drugs taken as control. Moreover, post simulation analysis of Methyl stearate complex predicted the most stable conformer. **Conclusions:** Further, *in-vivo* experiments are suggested to validate the medicinal use of Methyl stearate as potential inhibitors against spike protein of SARS-CoV-2.

INTRODUCTION

COVID-19 pandemic is caused by the novel coronavirus SARS-CoV-2, which is a member of the Coronaviridae family in the Nidovirales order [1]. The virus was first identified in Wuhan, China in late 2019 and has since spread globally, leading to widespread illness and death [2]. Coronaviruses are responsible for a range of diseases, including respiratory, digestive, enteric, and neurological disorders [3]. The highly transmissible nature of the virus has resulted in its spread to 216 countries worldwide [4]. As of the latest reported figures, there have been 759,408,703 confirmed cases of COVID-19 and 6,866,434 deaths attributed to the virus on a global scale according to WHO. The name coronavirus was derived from the crown-like appearance of spike protein on the surface of viral envelope [5]. The genome contains positive sense single-stranded RNA of 26-32 kilobases in length with the size ranges from 65-125 nanometers in diameter [6, 7]. Spike protein of SARS-CoV-2 facilitates the entry in host cell by interacting its glutamine residue at 394 positions in RBD domain with the lysine 31 residue on the human ACE2 receptor [8]. Spike protein is type 1 transmembrane fusion protein and is highly glycosylated [9, 10]. S1 (N-terminal)

DOI: https://doi.org/10.54393/pbmj.v6i3.853

subunit of Spike protein makes circular head of S protein, and second functional subunit is S2 (C-terminal) subunit that shapes the end of the protein and is embedded in viral envelope [11]. In viral-host cell interaction primarily S1 subunit identify and attach to the receptor present on the host cell and then S2 subunit, a highly well-conserved part assist in virus attachment to the host cellular membrane [12]. Due to the severity of this critical situation and the absence of a specific treatment, the scientific community and researchers are trying to find potential therapeutic agents that could be more effective in curing COVID-19. In this effort, scientists are also reviewing a large number of herbal plant species to identify active drug compounds [13]. The identification and efficacy of medicinal components in these plants could pave the way for combating COVID-19. The medicinal properties of *N. sativa*, a widely known herb, has potential in treating COVID-19 [14]. In the present study, in-silico method was used to screen *N. sativa* to identify its potential therapeutic compounds that can inhibit the SARS-CoV-2 infectious cycle. The identified compounds were then compared with clinically proposed drugs for COVID-19, including Arbidol, Favipiravir, Remdesivir, Nelfinavir, Chloroquine, and Hydroxychloroquine [15]. The study was performed using molecular docking and dynamic simulation. MOE is a drug discovery software that involves visualization, modelling, simulations, and methodology development. The physicochemical and drug likeness properties of the ligands were determined using various tools, including SwissADME, AdmetSAR, and Pfizer's rule of five.

METHODS

Dataset preparation

The two datasets of compounds from *N. sativa* having antiviral properties and commercially available assisting drug against COVID-19 taken as control was prepared. The viral spike protein against which the in-silico therapy has to propose was selected based on its primary role in onset of viral attachment and infection. The library was prepared by retrieving structures from PubChem and PDB (ID: 7BZ5) and perform preparatory changes to make ligand/protein suitable for docking analysis. The missing hydrogen bonds, charges were adjusted along with removal of repeated chains, heteroatoms, water molecules and already attached ligands. The energy minimization of ligands and protein were done by universal force field (UFF) with conjugate gradient algorithm of 500 iteration and Chimera using AMBER forcefield (AMBER ff14SB) respectively. The chemical compounds of *N. sativa* are given in Supplementary Table S1, while properties of protein are given in table 1.

Table 1: Crystallographic Properties of Spike Protein of SARS-CoV-2

Protein	PDB code	Classification	Organism	Expression system	Resolution	Method	Total structure Weight (DA)	Chain
Spike (S1)	7BZ5	Viral protein	SARS-CoV-2	Homo sapiens	1.84A	X-RAY Diffraction	73.36kDa	A

Virtual library screening of *N. sativa* against SARS-CoV-2 spike protein

The selected datasets were virtually screened for minimum binding energy with viral protein by docking with MOE. The ligands are allowed to interact with protein by creating a grid box with dimension of 20×20×20 Å around the active site of protein. The active site of spike protein was retrieved from previously published literature. MOE compute binding energy by calculating difference between the sum of energy in free state of ligand and protein and sum of energy in protein-ligand complex using Merck Molecular Force Field (MMFF). The binding energy (ΔG) of protein-ligand interactions was calculated using following empirical equation [16]. $\Delta G = (V^{L\text{-}L}_{bound} - V^{L\text{-}L}_{unbound}) + (V^{P\text{-}P}_{bound} - V^{P\text{-}P}_{unbound}) + (V^{P\text{-}L}_{bound} - V^{P\text{-}L}_{unbound} + \Delta S_{conf})$ where *P* refers to the protein, *L* refers to the ligand, $V^{L\text{-}L}_{bound}$ energy in bounded state of ligand, $V^{L\text{-}L}_{unbound}$ energy in unbounded state of ligand, $V^{P\text{-}P}_{bound}$ energy in bounded state of protein, $V^{P\text{-}P}_{unbound}$ energy in unbounded state of protein, $V^{P\text{-}L}_{bound}$ energy in bounded state of protein and ligand, $V^{P\text{-}L}_{unbound}$ energy in unbounded state of protein and ligand, ΔS_{conf} denotes the loss of conformational entropy upon binding.

Calculation of properties using ADMET analysis

AdmetSAR and Swiss ADME were used to analyzed drug-likeness of selected *N. sativa* compounds by computing pharmacokinetic and pharmacological properties.

Molecular dynamic simulations of the top-scoring ligand-protein complex

The Nanoscale Molecular Dynamics (NAMD) was used to perform molecular dynamic (MD) simulation to examine protein stability, conformational changes of ligand-protein complex, kinetics and free binding energy changes by allowing to interact in virtual environment similar to in-vivo condition. Ligands that were scrutinized through docking and drug likeliness analysis were selected for further MD simulation. The protein-ligand complex was prepared in same orientation with maximum score. The topologies of ligand and protein were made by Visual Molecular Dynamics (VMD) to define bonds/angles, number of molecules and atom types. The simulation inputs of ligand and protein were built from CHARMM-GUI web server with CHARMM36 forcefield and protein structure format generator of VMD respectively. Solvation box was made around a complex to provide medium and energy minimization was performed using conjugate gradient method. The periodic boundary conditions were adjusted with 310K temperature and 1 atm

DOI: https://doi.org/10.54393/pbmj.v6i3.853

pressure for simulation procedure. The MD simulation was executed for 1ns (500000 steps) using NAMD software after adjusting all parameters. Afterwards, the results were analyzed by plotting histogram of RMSD, hydrogen bond and heat map.

RESULTS

Molecular docking scores with clinical drugs

Clinical drugs used against COVID-19 were docked with spike protein and taken their results as control to compare with the compounds of *N. sativa*. Remedsivir, Nelfinavir and Chloroquine gave the lowest binding scores of -7.9, -7.2 and -7.2 Kcal/mol respectively. The docking scores of clinical drugs are listed in table 2.

Table 2: Docking scores of commercially available drugs against spike protein

Ligands	Docking Score (Kcal/mol)
Arbidol	-7.2468
Favipiravir	-4.3916
Remdesivir	-7.9584
Nelfinavir	-7.2471
Chloroquine	-6.4304
Hydroxychloroquine	-7.0793

Docking scores of *N. sativa* compounds

Molecular docking was performed between Spike Protein (ID: 7BZ5) of SARS-CoV-2 and Forty-six compounds of *N. sativa* separately for estimation of possible interactions between ligand and protein. The more negative binding energy indicates a stronger binding interaction. The binding energies of *N. sativa* compounds are given in Supplementary Table S2 while compounds with highest docking scores are listed in Table 3 with Methyl stearate binding energy of -7.4Kcal/mol.

Table 3: The best docked compounds of *N sativa* with spike protein

Ligands	Docking Score (Kcal/mol)
Methyl stearate	-7.4506
Eicosadienoic acid	-7.4234
Oleic acid	-7.1822
Stearic acid	-7.0729
Linoleic acid	-6.9182
Myristoleic acid	-6.4648
Palmitic acid	-6.3564
Farnesol	-6.1679

2D/3D interactions of best docked complexes having lowest binding energies are shown in Figure 1. It displays the spatial orientation of ligand in the binding pocket of protein with interaction to its surrounding amino acids.

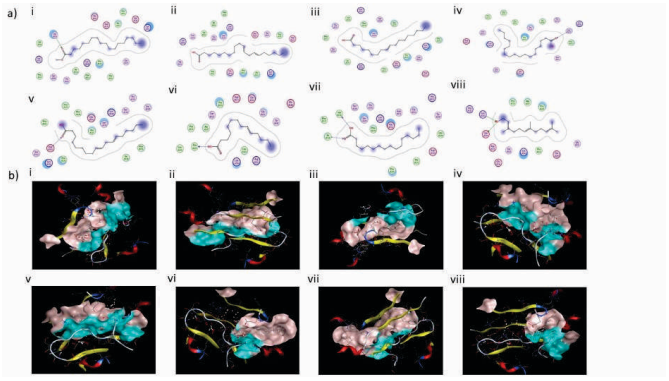

Figure 1: 2D/3D interaction between ligands of N sativa and spike protein.

a) 2D-interaction of complexes i) Methyl stearate with amino acid SER129 as hydrogen acceptor, distance/energy of 2.95Å/-1.3kcal/mol ii) Eicosadienoic acid with electrostatics interaction iii) Oleic acid with amino acid ASP123 as hydrogen donor, distance/energy of 2.81Å/-5.2kcal/mol iv) Stearic acid with amino acid SER209 as hydrogen acceptor, distance/energy of 3.11Å/-2.0kcal/mol v) Linoleic acid with amino acid ASP123 as hydrogen acceptor, distance/energy of 2.93Å/-4.2kcal/mol vi) Myristoleic acid with amino acid PRO125 as hydrogen donor, distance/energy of 2.83Å/-3.8kcal/mol vii) Palmitic acid with amino acid VAL116/ILE118 as hydrogen donor/acceptor, distance 3.35Å/3.18Å and energy -0.8/-1.1kcal/mol viii) Farnesol with amino acid GLU124/LYS211 as hydrogen donor/acceptor, distance 3.13Å/3.09Å and energy -1.3/-4.4kcal/mol b) 3D-interaction of complexes i) Methyl stearate ii) Eicosadienoic acid iii) Oleic acid iv) Stearic acid v) Linoleic acid vi) Myristoleic acid vii) Palmitic acid viii) Farnesol

Drug likeliness analysis

Compounds with highest scores were further analyzed for pharmacological properties by using AdmetSAR and Swiss ADME. This analysis predicted that all compounds follow Lipinski rule of five with only one violation which is acceptable for drug likeliness. The properties of blood-brain permeability and percentage of oral absorption in humans were calculated and found to be within the acceptable limits required for human usage (Table. 4).

Table 4: Lipinski's physiochemical guidelines for *N. sativa* compounds

Ligands	Molecular weight (g/mol)	H-Donor	H-Acceptor	Log p	Log S	TPSA (Å²)	Follow Lipinski's/ Violations
Methyl stearate	298.51	0	2	6.42	-3.399	26.30	Yes/1
Eicosadienoic acid	308.51	1	1	6.66	-4.04	37.30	Yes/1
Oleic acid	282.47	1	1	6.11	-4.04	37.30	Yes/1
Stearic acid	284.48	1	1	6.33	-3.502	37.30	Yes/1
Linoleic acid	280.45	1	1	5.88	-4.04	37.30	Yes/1
Myristoleic acid	226.36	1	1	4.55	-3.791	37.30	Yes/0
Palmitic acid	256.43	1	1	5.55	-3.502	37.30	Yes/1
Farnesol	222.37	1	1	4.40	-2.472	20.23	Yes/0

DOI: https://doi.org/10.54393/pbmj.v6i3.853

Molecular dynamic simulation analysis

Methyl stearate that gave the best docking score and have pharmacological properties was selected for MD simulation to investigate its time-dependent binding ability and conformational stability in spike protein binding pocket. Although molecular docking determines the three-dimensional orientation of ligand within the receptor pocket with minimum energy further conformational stability is necessary to assess the inhibitory strength of compounds against spike protein. Histogram of root mean square deviation (RMSD), hydrogen bonds and heat map are plotted to analyze the results.

RMSD analysis

Root mean square deviation (RMSD) was calculated to determine the average distance between atom groups. The RMSD plot indicated that the Methyl stearate-spike protein complex remained stable for almost whole time of molecular dynamic simulation with high stability shown at 1.5 Å from 60 to 125 frames. Afterwards, the RMSD value slightly increased to 1.7 Å from 125 to 200 frames (Figure 2).

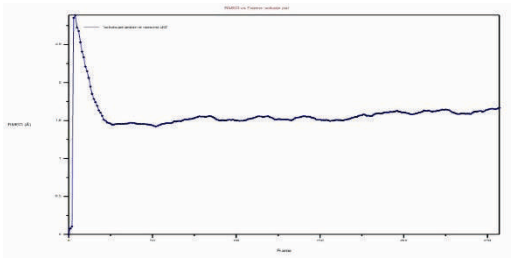

Figure 2: The RMSD graph of Methyl stearate complex with spike protein

Analysis of hydrogen bonds

The stability of complexes was investigated by hydrogen bond analysis as formation of strong hydrogen bond reduces the gap between residues and therefore increases the stability of complex. Hydrogen bond analysis showed that the major bond is formed between SegHP1-GLY44 (donor) and SegLIG-LIG1 (acceptor) with an occupancy rate of 26.74%. The H-bond graphs of Methyl stearate is shown in Figure 3.

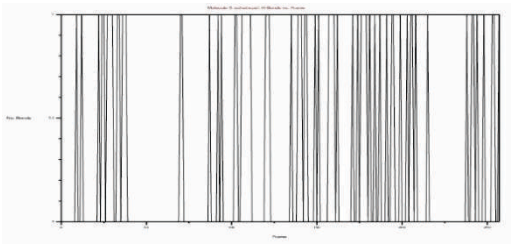

Figure 3: The histogram of H-bond analysis of Methyl stearate complex with spike protein

Complex analysis using heat maps

Heat map plots display how a specific characteristic, such as potential energy, temperature, pressure, or density, of a simulated system is spread out over time. The heat signature of Methyl stearate-spike protein complex showed quite stability and given in Figure 4.

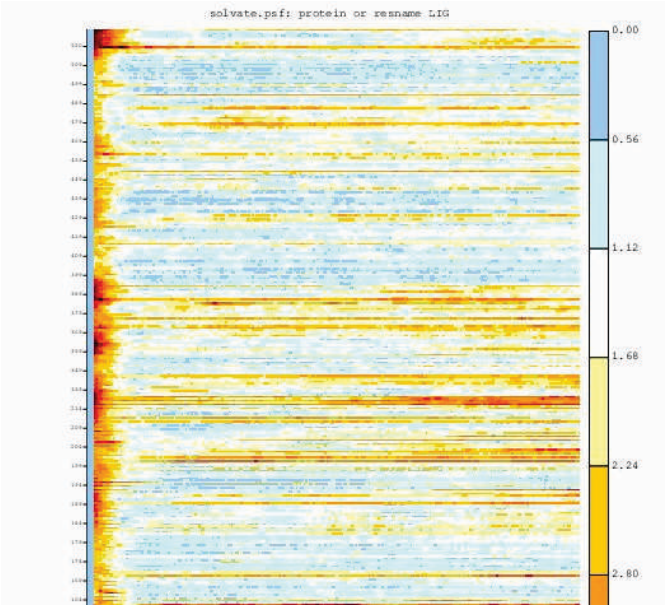

Figure 4: The heatmap graph of Methyl stearate complex with spike protein

DISCUSSION

The global health crisis caused by the COVID-19 pandemic had drastic effects on individuals around the world and is widely recognized as a major threat to public health. Despite the absence of an approved drug, efforts are underway in various areas of medicine, such as allopathic and homeopathic to find a solution of the problem. The high mutation rate of the RNA genome is a significant obstacle in drug discovery, as it can lead to reduced effectiveness of drugs [17]. This is a major limitation that researchers must overcome in order to develop more effective treatments for diseases caused by RNA viruses such as COVID-19. The study aimed to evaluate the potential of therapeutic drug agents derived from bioactive compounds of *Nigella sativa* using molecular docking and dynamic simulation techniques against the spike protein of SARS-CoV-2, which is considered a potential target for drug development and effective treatments for COVID-19. The structures of total 46 *N. sativa* compounds were identified and retrieved from literature and databases respectively. Their binding energies with the target protein were calculated through docking using MOE. The stability and drug likeliness of ligands which presented lowest binding energies with viral protein was further evaluated through MDS and ADMET analysis. The *in-silico* analysis predicted Methyl stearate as the potential inhibitor. Similar studies by Saif *et al.*, have been reported in the past in predicting the potential inhibitor of this virus. Recent studies predicted the

DOI: https://doi.org/10.54393/pbmj.v6i3.853

promising inhibitor against main protease and spike protein from the compounds of *Olea europaea*, *Curcuma longa* and *Carica papaya* respectively [18, 19]. The present study was limited to computational analysis, as molecular dynamics simulations require significant computational power for extended periods of time. Therefore, more accurate assessments of the stability of these complexes over longer time frames would require higher computational system [20].

CONCLUSIONS

Current computer-aided drug designing (CADD) investigated Methyl stearate from *N. sativa* compounds as potential inhibitor of SARS-CoV-2 by demonstrating lowest binding energy and forming stable complex with viral spike protein. Further wet-experimental research is needed to validate its inhibitory effect on SARS-CoV-2 before clinical trials.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, *et al*. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology. 2020 Apr; 5(4): 536-44. doi: 10.1038/s41564-020-0695-z.

[2] Ng Y, Li Z, Chua YX, Chaw WL, Zhao Z, Er B, *et al*. Evaluation of the effectiveness of surveillance and containment measures for the first 100 patients with COVID-19 in Singapore—January 2–February 29, 2020. Morbidity and Mortality Weekly Report. 2020 Mar; 69(11): 307. doi: 10.15585/mmwr.mm6911e1.

[3] Kamble P, Daulatabad V, John N, John J. Synopsis of symptoms of COVID-19 during second wave of the pandemic in India. Hormone Molecular Biology and Clinical Investigation. 2021 Dec; 43(1): 97-104. doi: 10.1515/hmbci-2021-0043.

[4] Cvetković VM, Nikolić N, Radovanović Nenadić U, Öcal A, K. Noji E, Zečević M. Preparedness and preventive behaviors for a pandemic disaster caused by COVID-19 in Serbia. International Journal of Environmental Research and Public Health. 2020 Jun; 17(11): 4124. doi: 10.3390/ijerph17114124.

[5] Coutard B, Valle C, De Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Research. 2020 Apr; 176: 104742. doi: 10.1016/j.jare.2020.03.005.

[6] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Emergence, transmission, and characteristics of human coronaviruses. Journal of Advanced Research. 2020 Jul; 24: 91-8. doi: 10.1016/j.jare.2020.03.005.

[7] Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. Journal of Pharmaceutical Analysis. 2020 Apr; 10(2): 102-8. doi: 10.1016/j.jpha.2020.03.001.

[8] Gyebi GA, Adegunloye AP, Ibrahim IM, Ogunyemi OM, Afolabi SO, Ogunro OB. Prevention of SARS-CoV-2 cell entry: insight from in silico interaction of drug-like alkaloids with spike glycoprotein, human ACE2, and TMPRSS2. Journal of Biomolecular Structure and Dynamics. 2022 Mar; 40(5): 2121-45. doi: 10.1080/07391102.2020.1835726.

[9] Shajahan A, Supekar NT, Gleinich AS, Azadi P. Deducing the N-and O-glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2. Glycobiology. 2020 Dec; 30(12): 981-8. doi: 10.1093/glycob/cwaa042.

[10] Li X, Luk HKH, Lau SKP, Woo PCY. Human Coronaviruses: General Features. Reference Module in Biomedical Sciences. 2019: B978-0-12-801238-3.95704-0. doi: 10.1016/B978-0-12-801238-3.95704-0.

[11] Yang H and Rao Z. Structural biology of SARS-CoV-2 and implications for therapeutic development. Nature Reviews Microbiology. 2021 Nov; 19(11): 685-700. doi: 10.1038/s41579-021-00630-8.

[12] Iqbal Yatoo M, Hamid Z, Parray OR, Wani AH, Ul Haq A, Saxena A, *et al*. COVID-19-Recent advancements in identifying novel vaccine candidates and current status of upcoming SARS-CoV-2 vaccines. Human Vaccines & Immunotherapeutics. 2020 Dec; 16(12): 2891-904. doi: 10.1080/21645515.2020.1788310.

[13] Sytar O, Brestic M, Hajihashemi S, Skalicky M, Kubeš J, Lamilla-Tamayo L, *et al*. COVID-19 prophylaxis efforts based on natural antiviral plant extracts and their compounds. Molecules. 2021 Jan; 26(3): 727. doi: 10.3390/molecules26030727.

[14] Imran M, Khan SA, Alshammari MK, Alkhaldi SM, Alshammari FN, Kamal M, *et al*. *Nigella sativa* L. and COVID-19: A glance at the anti-COVID-19 chemical constituents, clinical trials, inventions, and patent literature. Molecules. 2022 Apr; 27(9): 2750. doi: 10.3390/molecules27092750.

[15] Bouchentouf S and Missoum N. Identification of Compounds from *Nigella Sativa* as New Potential Inhibitors of 2019 Novel Coronasvirus (Covid-19):

DOI: https://doi.org/10.54393/pbmj.v6i3.853

Molecular Docking Study. 2020 Apr: 1-12. doi: 10.26434/chemrxiv.12055716.v1.

[16] Dias R and de Azevedo WF Jr. Molecular docking algorithms. Currents Drug Targets. 2008 Dec; 9(12): 1040-7. doi: 10.2174/138945008786949432.

[17] Yin J, Li C, Ye C, Ruan Z, Liang Y, Li Y, *et al*. Advances in the development of therapeutic strategies against COVID-19 and perspectives in the drug design for emerging SARS-CoV-2 variants. Computational and Structural Biotechnology Journal. 2022 Jan; 20: 824-837. doi: 10.1016/j.csbj.2022.01.026.

[18] Saif R, Raza MH, Rehman T, Zafar MO, Zia S, Qureshi AR. Molecular docking and dynamic simulation of Olea europaea and Curcuma Longa compounds as potential drug agents for targeting Main-Protease of SARS-nCoV2. Biological and Medicinal Chemistry. 2021 Mar: 1-22. doi: 10.26434/chemrxiv.13246739.v2.

[19] Saif R, Zafar MO, Raza MH, Zia S, Qureshi AR. Computational prediction of Carica papaya phytocompounds as potential drug agent against RdRp and spike protein of SARS-nCoV2 by molecular docking and dynamics simulation approaches. Research Square. 2022 Aug. doi: 10.21203/rs.3.rs-105301/v2.

[20] Naqvi AA, Mohammad T, Hasan GM, Hassan M. Advancements in docking and molecular dynamics simulations towards ligand-receptor interactions and structure-function relationships. Current Topics in Medicinal Chemistry. 2018 Aug; 18(20): 1755-68. doi: 10.2174/1568026618666181025114157.

DOI: https://doi.org/10.54393/pbmj.v6i3.856

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 3 (March 2023)

Original Article

Sedentary Lifestyle Associated Hyperventilation Syndrome among Students of Karachi Quarantined Amidst COVID Out Break: A Cross Sectional Survey

Anika Nadeem[1*], Ramesha Qazi[2], Anum Zafar[3], Sadia Batool[4] and Rizmi Naseer[2]

[1]Liaquat National School of Physiotherapy, Karachi, Pakistan
[2]Hamza Medical Centre, Islamabad, Pakistan
[3]Department of Rehablitation Sciences, Ibadat International University, Islamabad, Pakistan
[4]PainFix Physical Therapy Clinic, Abid Hospital, Islamabad, Pakistan

ARTICLE INFO

Key Words:
Hyperventilation Syndrome, Sedentary, Lifestyle, COVID, Quarantine

How to Cite:
Nadeem, A. . ., Qazi, R. ., zafar , A. ., Batool, S. ., & Naseer, R. . (2023). Sedentary Lifestyle Associated Hyperventilation Syndrome among Students of Karachi Quarantined Amidst COVID Out Break: A Cross Sectional Survey: Sedentary Lifestyle Associated Hyperventilation Syndrome. Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.856

***Corresponding Author:**
Anika Nadeem
Liaquat National School of Physiotherapy, Karachi, Pakistan
Anika.nadeem25@hotmail.com

Received Date: 2nd March, 2023
Acceptance Date: 28th March, 2023
Published Date: 31st March, 2023

ABSTRACT

Sedentary activity has been related to poor physical health outcomes in both adults and youth in previous studies. While there is growing evidence of a correlation between sedentary behavior and mental health outcomes, little is known about the risk of hyperventilation syndrome. **Objective:** To find out the prevalence of hyperventilation syndrome due to sedentary lifestyle among students of Karachi quarantined amidst COVID outbreak. **Methods:** A cross sectional online survey based questionnaire which included IPAQ (International physical activity questionnaire) to access sedentary behavior and Nijmegen questionnaire to rule out hyperventilation syndrome was used to collect data from 214 students from Karachi, Pakistan. **Results:** Out of the total 214 subjects, 128(59.5%) developed a sedentary behavior and 86(40%) were found non sedentary. These 128(59.5%) students were further assessed for hyperventilation out of which 114(53%) were found to be positive. **Conclusions:** The study concluded that 53% students of Karachi with sedentary lifestyle developed hyperventilation syndrome amidst COVID outbreak.

INTRODUCTION

Corona Virus is the novel and much stronger form of the virus SARS (severe acute respiratory syndrome) which occurred as a major outbreak in Hong Kong in March 2003 and is considered as the first global epidemic of the twenty-first century [1]. According to World Health Organization (WHO) more than 20 million people from over 200 countries across the world have been affected by this pandemic, resulting in over 730,000 deaths [2]. As a result, governments worldwide have made extraordinary attempts to control the disease, by ensuring methods for social distancing among general population and prescribing quarantine and isolation for people who have been tested positive for COVID-19 [2]. As the educational institutes have crowded environments, many countries have instructed to shift the traditional classroom education to online classes to keep the students safe at home yet not wasting their time either as it is not known when the pandemic would end. In 2019, Online education in many countries became compulsory as there was no choice left for the institutes due to the spread of Corona Virus. Although these steps are highly appreciable and vital for reducing the spread of COVID-19, they contributed to unhealthy habits, such as a sedentary lifestyle [2]. Sedentary lifestyle or sedentary behavior is described as

DOI: https://doi.org/10.54393/pbmj.v6i3.856

any non-sleep activity using minimum energy expenditure resulting in low energy expenditure comparable to the amount of resting [approximately 1.0 to 1.5 metabolic equivalent (METS)] [3, 4]. This involves activities such as sitting for a variety of reasons (e.g. work, travel), and screen based activities such as computer use, video gaming and watching television for longer period of times [3]. A sedentary lifestyle, including physical inactivity and extended sedentary behavior, has previously been described as problematic under normal circumstances with one third of population physical inactive and 41.5% spending four or more hours a day sitting worldwide [2]. Sedentary lifestyle can lead to mental health problems such as anxiety [5].Globally it is estimated that the prevalence of anxiety disorders is 7.3 and is commonly found in individuals of around 15-34 years of age. It was also found to be more prevalent in woman than in men [6]. Moreover, an overall negative effect on physical activity intensity was however observed during the pandemic [2, 7]. Sedentary behavior has also been linked to a range of negative health outcomes in a variety of young population in previous studies such as sleep problems, musculoskeletal pain, depression, poor psychological well-being, depression, bipolar disorder and schizophrenia including hyperventilation syndrome (HVS) [8-10]. HVS involves breathing too deeply or too rapidly and it might include pain in the chest, breathlessness if it is ignored and left untreated [10]. The symptoms might also include paresthesia, tightness in the chest and dizziness [11]. In other words, it also means breathing in surplus of metabolic requirements [12, 13]. This is demonstrated by an abnormal and disorganized breathing pattern identified as Tachypnoea [12]. The Nijmegen questionnaire put forward in 1980's is widely used today to diagnose hyperventilation syndrome. This is a 16- item screening technique to identify people who could benefit from breathing retraining. Panic, anxiety attack, anxiety condition, dysfunctional breathing and breathing pattern disorder are alternate words or terminologies used in literature [12]. However, there is insufficient evidence on the relation between sedentary lifestyle and HVS. This study aims to analyze relationship between sedentary lifestyle and Hyperventilation syndrome among students of Karachi quarantined during COVID-19 outbreak.

METHODS

A cross sectional survey based online research was conducted over a period of 4 months from September 2020 to December 2020, the study was conducted on males and females between the age of 15 to 26 living in Karachi, sample size was calculated using slovin formula and sample of 214 was selected from based of non-probability convenient sampling. The participation was entirely voluntary and based on following inclusion criteria; who have signed consent form and are currently enrolled as regular students in degree program or higher secondary education. Any young adult suffering from any kind of respiratory condition, psychiatric disease or any other medical condition were excluded from the study Participants having medical condition like asthma, chronic obstructive pulmonary disease, pulmonary edema, were excluded from study as these diseases cause hyperventilation. Also, participants taking medication that may induce hyperventilation were excluded from the study. A survey was designed to complete in 4-5 minutes which included 3 sections. Section A was about demographic details which contained six questions on name, gender, age, city, email address and last question phrased "How much time did you overall spend sitting each day in the past 7 days" with options a. less than 4 hours, b. 4-8 hours, c. 8-11 hours, d. more than 11 hours. Section B and C contained measurement tools i.e. IPAQ (International physical activity questionnaire) to access sedentary behavior of an individual and hyperventilation syndrome was analyzed using Nijmegen Questionnaire in the last section. The IPAQ consists of 11 questions representing domain activities over the previous 7 days while Nijmegen Questionnaire was based on 16 symptoms. The form was circulated online through several online platforms and the data were collected through non-probability convenient based sampling. Students of both genders between 15-25 years of age who were living a sedentary lifestyle in Karachi during lockdown in COVID-19 were included in this study. Nijmegen questionnaire containing 16 symptoms is a standardized self-administered reported outcome measure which is widely used for ruling out hyperventilation syndrome. It can be rated on a five-point ordinal scale (0=NEVER, 1=RARE, 2=SOMETIMES, 3=OFTEN, 4=VERY OFTEN) according to the frequency of occurrence. If the score is 24 out of 64, HVS will be considered positive [14]. IPAQ is an internationally standardized self-report questionnaire that is used as a measuring tool for individuals between 15-69 years of age to measure and estimate the physical activity and sedentary behavior [15, 16]. We categorized sedentary behavior in 2 categories. A score of less than 15 was named non-sedentary and a score of 16-30 was considered positive for sedentary behavior. Collected data were analyzed using SPSS version 22.0 statistical software. The demographic profile of the participants is presented in form of frequency and percentage. The results were displayed in the form of cross tabulation, graphs and tables. Chi- square test was applied to assess the association of age and gender with sedentary lifestyle and hyperventilation syndrome. Correlation between sedentary lifestyle and hyperventilation syndrome was also

DOI: https://doi.org/10.54393/pbmj.v6i3.856

observed using Spearman's correlation coefficient test.

RESULTS

There was a total of 214 participants among which 27(13%) were males and 187(87.0%) were females (Figure 1).

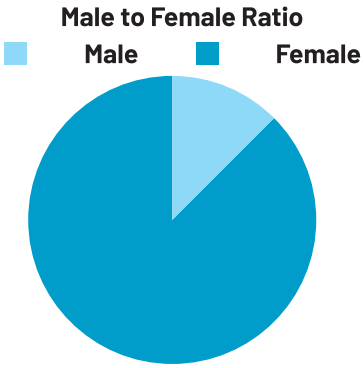

Figure 1: Representing Male to Female ratio

Figure 2 shows age range of participants. 86 participants were from age 23-24, 29 were of age 15-16, 29 were of age 25-26, 24 were of age 19-20 and 21-22 and 22 were of age 17-18.

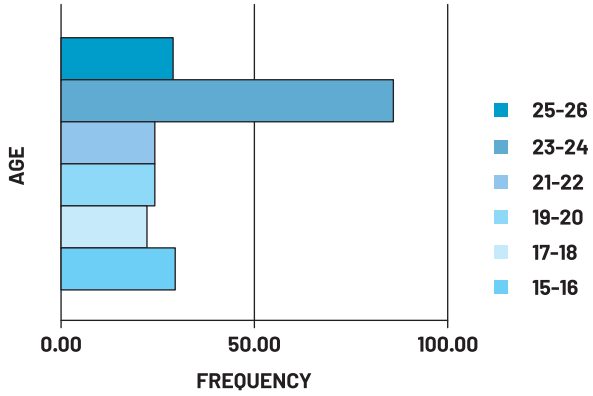

Figure 2: Distribution of ages by graphical representation

90.9% of students enrolled were from Karachi who were included in this research study and 9.1% were from another city that were excluded. Frequency (N) and percentage (%) of both sedentary lifestyle and hyperventilation syndrome was assessed (Table 1). 59.5% of our population was found sedentary out of which 53% had hyperventilation syndrome.

Table 1: Distribution of responses of the questionnaire

Questions	Not at all	Less than 1 hour	1-2 Hour	3-4 Hour	More than 4 hour
	N(%)	N(%)	N(%)	N(%)	N(%)
Watching TV	31(14.4)	33(15.3)	67(31.2)	49(22.8)	34(15.8)
Watching Videos/DVDS	24(11.2)	37(17.2)	67(31.2)	52(24.2)	34(15.8)
Computer for fun	49(22.8)	39(18.1)	84(39.1)	27(12.6)	15(17.0)
Computer for homework	39(18.1)	51(23.7)	89(41.4)	26(12.1)	9(4.2)
Doing Homework	56(26.0)	54(25.1)	76(35.3)	18(84)	10(4.7)
Reading for fun	48(22.3)	89(41.4)	56(26.0)	14(6.5)	7(3.3)
Being tutored	86(40.0)	49(22.8)	47(21.9)	23(10.7)	9(4.2)
Travel	48(22.3)	99(46.0)	52(24.2)	11(5.1)	4(1.9)
Crafts & Hobbies	61(28.4)	65(30.2)	55(25.6)	26(12.1)	7(3.3)
Sitting around	19(8.8)	32(14.9)	53(24.7)	48(22.3)	62(28.8)
Practice musical instrument	110(51.2)	66(30.7)	31(14.4)	6(2.8)	1(5)
Part B: Nigmegan Questionnaire for Hyperventilation Syndrome					
Questions	**Not at all**	**Less than 1 hour**	**1-2 Hour**	**3-4 Hour**	**More than 4 hour**
	N(%)	**N(%)**	**N(%)**	**N(%)**	**N(%)**
Chest Pain	66(30.7)	53(24.7)	43(20.0)	19(8.8)	0(0)
Felling Tense	18(8.4)	43(20.0)	85(39.5)	28(13.0)	7(3.3)
Blurred Vision	60(27.9)	27(12.6)	70(32.6)	21(9.8)	3(1.4)
Dizzy Spells	50(23.3)	60(27.9)	55(25.6)	12(5.6)	4(1.9)
Feeling Confused	30(14.0)	52(24.2)	76(35.3)	17(7.9)	6(2.8)
Faster Or Deeper Breathing	49(22.8)	56(26.0)	59(27.4)	15(7.0)	2(9)
Shortness Of Breath	68(31.6)	43(20.0)	53(24.7)	14(6.5)	3(1.4)
Tight Feeling in Chest	72(33.5)	61(28.4)	42(19.5)	5(2.3)	1(5)
Bloated Feeling in Stomach	45(20.9)	55(25.6)	51(23.7)	22(10.2)	8(3.7)
Tingling Fingers	66(30.7)	58(27.0)	37(17.2)	14(6.5)	6(2.8)
Unable To Breathe Deeply	64(29.8)	52(24.2)	50(23.3)	7(3.3)	8(3.7)
Stiff Fingers or Arms	68(31.6)	47(21.9)	43(20.0)	8(3.7)	15(70)
Tight Feeling Around Mouth	83(38.6)	45(20.9)	37(17.2)	5(23)	11(51)
Cold Hands or Feet	36(16.7)	57(26.5)	67(31.2)	9(4.2)	12(5.6)
Palpitation (pounding heart)	54(25.1)	46(21.4)	44(20.5)	23(10.7)	14(6.5)
Feeling Of Anxiety	32(14.9)	43(20.0)	53(24.7)	37(17.2)	16(7.4)

Results of the study show a majority number of students spent more time sitting during lockdown with 33(15%) sitting for less than 4 hours a day, 75(35%) for 4-8 hours, 85 (40%) for 8-11 hours and 21(10%) spent more than 11 hours sitting each day (Figure 3).

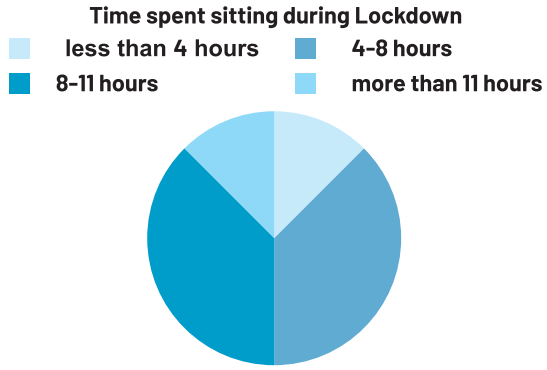

Figure 3: Graphical representation of time spent sitting during lockdown

Table 2 shows prevalence of hyperventilation syndrome in participants. 62 (28.2%) were negative while 114 (53.0%) were positive.

Table 2: Prevalence of hyperventilation syndrome

Hyperventilation syndrome (HVS)	Frequency (%)
Negative	62(28.2)
Positive	114(53.0)
Total	176(81.9)

Figure 4 shows prevalence of Hyperventilation Syndrome (HVS) in graphical manner.

Figure 4: Graphical representation of prevalence of

DOI: https://doi.org/10.54393/pbmj.v6i3.856

hyperventilation syndrome

Chi- square test was applied to assess the association of age and gender with sedentary lifestyle and hyperventilation syndrome. A p-value of < 0.05 was considered statistically significant (Table 3).

Table 3: Chi-Square Test

	IPAQ (International Physical Activity Questionnaire)				NIJMEGAN Questionnaire			
	Non sedentary	Sedentary	Total	p-value	Negative	Positive	Total	p-value
Gender								
Male	18	9	27	0.003	13	61	27	0.003
Female	68	119	187		49	08	187	
Gender								
15-16	15	14	29	0.704	5	14	29	0.226
17-18	7	15	22		7	11	22	
19-20	9	15	24		7	15	24	
21-22	11	13	24		8	9	24	
23-24	32	54	86		25	49	86	
25-26	12	17	29		10	16	29	
Total	86	128	214		62	114	214	

p-value < 0.05 taken as statistically significant

Correlation between sedentary lifestyle and hyperventilation syndrome was also observed. A strong correlation was found between sedentary lifestyle and risk of hyperventilation syndrome. Spearman's correlation coefficient test was used where 'rho' was 0.765 indicating a positive linear relationship between both variables. A *p*-value of < 0.01 was taken as statistically significant (Table 4).

Table 4: Correlation between sedentary lifestyle and hyperventilation syndrome

	Hyperventilation syndrome (HVS)	Sedentary Lifestyle (S)
(HVS) Spearman's rho	1	0.765**
Sig (2-tailed)	-	0.000
N	214	214
(S) Spearman's rho	0.765**	1
Sig (2-tailed)	0.000	-
N	214	214

** Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

According to the results of this study 128(59.5%) students including male and female developed a sedentary lifestyle during lockdown and 86(40%) remained non-sedentary. This was assessed using IPAQ (International Physical Activity Questionnaire) which is a standardized measuring tool used to measure and estimate the physical activity and sedentary behavior [15, 16]. 128 students who were found living a sedentary lifestyle were further assessed for the risk of hyperventilation syndrome due to sedentary behavior. It was found that out of 128 students 114(53%) showed symptoms of positive hyperventilation syndrome while results of 62 (28.2%) came negative for hyperventilation syndrome (as shown in table 2). According to the results of this research study students spent more time sitting than doing physical activities. This finding was hypothesized by some studies during beginning of COVID pandemic. A study by Margaritis I hypothesized that during pandemic overall physical activity will decrease and sedentary behavior increase causing exposure risk factors known for its relation to insufficient physical activity [17]. The results are similar to finding of other studies, A study conducted by Romero-Blanco C *et al.*, to evaluate students' physical activity and sedentary behavior before and during the coronavirus lockdown. Using the International Physical Activity Questionnaire-Short Form (IPAQ-SF) showed increase in both weekly physical activity and sitting time During lockdown. However, group analysis indicated difference in relation to gender, year of study, BMI, alcohol consumption, tobacco use, symptoms of anxiety/depression, Mediterranean diet, living situation and stage of change [18]. The initial hypothesis was partially verified in this research i.e. the amount of time spent sitting and sedentary behavior of students have increased during lockdown as sedentary behavior patterns are influenced by the environment in which a person lives. However, as our data weren't distributed in both genders equally, we cannot claim if female students had a more sedentary behavior than male students during COVID-19 lockdown or vice versa. Further research is needed to study this relation [19]. The current body of evidence on the relationship between sedentary behavior and hyperventilation syndrome is limited according to the literature review, although studies are present associating sedentary behavior with other medical conditions including psychosis [20], depression [21], physical activity [5, 17], stress and anxiety [22]. Anxiety and panic attack are the terms used in the literature referring hyperventilation syndrome [12]. A study conducted by Motiejunaite *et al.*, to assess the possible relationship between HVS and previous acute COVID-19 infection through a large-scale cross-sectional single center study using a systemic Nijmegen questionnaire and a standardized lung function test by dividing population according to HVS diagnosis, defined as a Nijmegen score of > 23/64. The occurrence of previous COVID-19 infection was compared according to the Nijmegen score after adjustment for potential confounders by multivariate logistic regression. The results of this large-scale, cross-sectional study suggest an association between HVS diagnosis and a history of COVID-19 disease in patients who were not hospitalized [23]. In this study association of sedentary behavior and hyperventilation with age and gender was determined using Chi square test (as shown in Table 3). The results show a strong relationship of gender with sedentary lifestyle and hyperventilation

DOI: https://doi.org/10.54393/pbmj.v6i3.856

syndrome (p-value 0.003). Similar results are found in literature as a study conducted by Taverne *et al.*, where post COVID patients were clinically evaluated for 3 months also observed female predominance in idiopathic hyperventilation syndrome [24]. No relation of age was found with sedentary lifestyle and hyperventilation syndrome.

CONCLUSIONS

In conclusion, this study provides evidence that the COVID-19 pandemic and resulting lockdown have led to a significant increase in sedentary behavior among young students in Karachi. The findings also suggest that sedentary behavior is strongly associated with hyperventilation syndrome, with over half of the students who developed a sedentary lifestyle during lockdown showing symptoms of the condition.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Lau AL, Chi I, Cummins RA, Lee TM, Chou KL, Chung LW. The SARS (severe acute respiratory syndrome) pandemic in Hong Kong: Effects on the subjective wellbeing of elderly and younger people. Aging and Mental Health. 2008 Nov; 12(6): 746-60. doi: 10.1080/13607860802380607.

[2] Zheng C, Huang WY, Sheridan S, Sit CH, Chen XK, Wong SH. COVID-19 pandemic brings a sedentary lifestyle in young adults: a cross-sectional and longitudinal study. International Journal of Environmental Research and Public Health. 2020 Sep; 17(17): 6035. doi: 10.3390/ijerph17176035.

[3] Teychenne M, Costigan SA, Parker K. The association between sedentary behaviour and risk of anxiety: a systematic review. BMC Public Health. 2015 Dec; 15(1): 1-8. doi: 10.1186/s12889-015-1843-x.

[4] Pate RR, Stevens J, Webber LS, Dowda M, Murray DM, Young DR, *et al*. Age-related change in physical activity in adolescent girls. Journal of Adolescent Health. 2009 Mar; 44(3): 275-82. doi: 10.1016/j.jadohealth.2008.07.003.

[5] Lee E and Kim Y. Effect of university students' sedentary behavior on stress, anxiety, and depression. Perspectives in Psychiatric Care. 2019 Apr; 55(2): 164. doi: 10.1111/ppc.12296.

[6] Allen MS, Walter EE, Swann C. Sedentary behaviour and risk of anxiety: a systematic review and meta-analysis. Journal of Affective Disorders. 2019 Jan; 242: 5-13. doi: 10.1016/j.jad.2018.08.081.

[7] Ammar A, Brach M, Trabelsi K, Chtourou H, Boukhris O, Masmoudi L, *et al*. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. Nutrients. 2020 May; 12(6): 1583. doi: 10.3390/nu12061583.

[8] Williams CY, Williams RW, Knight R, Hashmi S, Donnelly N, Bance M. Hyperventilation Syndrome: Investigating the Relationship Between Nijmegen Questionnaire, Vestibular Function Tests, and Patient Symptoms. Otology & Neurotology. 2020 Mar; 41(3): e349-56. doi: 10.1097/MAO.0000000000002531.

[9] Costigan SA, Barnett L, Plotnikoff RC, Lubans DR. The health indicators associated with screen-based sedentary behavior among adolescent girls: a systematic review. Journal of Adolescent Health. 2013 Apr; 52(4): 382-92. doi: 10.1016/j.jadohealth.2012.07.018.

[10] Barker NJ, Jones M, O'Connell NE, Everard ML. Breathing exercises for dysfunctional breathing/hyperventilation syndrome in children. Cochrane Database of Systematic Reviews. 2013 Dec; 2013(12): CD01037. doi: 10.1002/14651858.CD010376.pub2.

[11] Jones M, Harvey A, Marston L, O'Connell NE. Breathing exercises for dysfunctional breathing/hyperventilation syndrome in adults. Cochrane Database of Systematic Reviews. 2013 May; 2013(5): CD0090. doi: 10.1002/14651858.CD009041.pub2.

[12] Wilson C. Hyperventilation syndrome: diagnosis and reassurance. Journal of Pandemic Practice. 2018; 10(9): 370-5. doi: 10.12968/jpar.2018.10.9.370.

[13] Folgering H. The pathophysiology of hyperventilation syndrome. Monaldi archives for chest disease= Archivio Monaldi per le Malattie Del Torace. 1999 Aug; 54(4): 365-72.

[14] Li Ogilvie YM. Validity of the nijmegen questionnaire for hyperventilation syndrome (Doctoral dissertation, Auckland University of Technology). 2017. Available at:https://openrepository.aut.ac.nz/items/383881a0-38af-418d-9be5-a4f558ac3f92.

[15] Cleland C, Ferguson S, Ellis G, Hunter RF. Validity of the International Physical Activity Questionnaire (IPAQ) for assessing moderate-to-vigorous physical activity and sedentary behaviour of older adults in the United Kingdom. BMC Medical Research Methodology. 2018 Dec; 18: 1-2. doi: 10.1186/s12874-018-0642-3.

[16] International Physical Activity Questionnaire.

DOI: https://doi.org/10.54393/pbmj.v6i3.856

Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ)-short and long forms. 2005. Available at: https://www.researchgate.net/file.PostFileLoader.html?id=5641f4c36143250eac8b45b7&assetKey=AS%3A294237418606593%401447163075131

[17] Margaritis I, Houdart S, El Ouadrhiri Y, Bigard X, Vuillemin A, Duché P. How to deal with COVID-19 epidemic-related lockdown physical inactivity and sedentary increase in youth? Adaptation of Anses' benchmarks. Archives of Public Health. 2020 Dec; 78: 1-6. doi: 10.1186/s13690-020-00432-z.

[18] Romero-Blanco C, Rodríguez-Almagro J, Onieva-Zafra MD, Parra-Fernández ML, Prado-Laguna MD, Hernández-Martínez A. Physical activity and sedentary lifestyle in university students: changes during confinement due to the COVID-19 pandemic. International Journal of Environmental Research and Public Health. 2020 Sep; 17(18): 6567. doi: 10.3390/ijerph17186567.

[19] Cheval B, Sivaramakrishnan H, Maltagliati S, Fessler L, Forestier C, Sarrazin P, *et al.* Relationships between changes in self-reported physical activity, sedentary behaviour and health during the coronavirus (COVID-19) pandemic in France and Switzerland. Journal of Sports Sciences. 2021 Mar; 39(6): 699-704. doi: 10.1080/02640414.2020.1841396.

[20] Stubbs B, Williams J, Gaughran F, Craig T. How sedentary are people with psychosis? A systematic review and meta-analysis. Schizophrenia Research. 2016 Mar; 171(1-3): 103-9. doi: 10.1016/j.schres.2016.01.034.

[21] Zhai L, Zhang Y, Zhang D. Sedentary behaviour and the risk of depression: a meta-analysis. British Journal of Sports Medicine. 2015 Jun; 49(11): 705-9. doi: 10.1136/bjsports-2014-093613.

[22] Peterson NE, Sirard JR, Kulbok PA, DeBoer MD, Erickson JM. Sedentary behavior and physical activity of young adult university students. Research in Nursing & Health. 2018 Feb; 41(1): 30-8. doi: 10.1002/nur.21845.

[23] Motiejunaite J, Balagny P, Arnoult F, Mangin L, Bancal C, Vidal-Petiot E, *et al.* Hyperventilation as one of the mechanisms of persistent dyspnoea in SARS-CoV-2 survivors. European Respiratory Journal. 2021 Aug; 58(2): 2101578. doi: 10.1183/13993003.01578-2021.

[24] Taverne J, Salvator H, Leboulch C, Barizien N, Ballester M, Imhaus E, *et al.* High incidence of hyperventilation syndrome after COVID-19. Journal of Thoracic Disease. 2021 Jun; 13(6): 3918. doi: 10.21037/jtd-20-2753.

DOI: https://doi.org/10.54393/pbmj.v6i3.897

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 3 (March 2023)

Original Article

Renal Toxicity Induced by Carbon Tetrachloride in Experimental Model and between authors

Mirza Fahad Baig[1], Muhammad Khalil Ahmad Khan[1*]

[1]Department of Zoology, University of Okara, Renalla Khurd, Pakistan

ARTICLE INFO

Key Words:
CCl4, Renal Toxicity, Biochemical Parameters

How to Cite:
Baig, M. F., & Ahmad Khan, M. K. . (2023). Renal Toxicity Induced by Carbon Tetrachloride in Experimental Model: Renal Toxicity Induced by CCl4 in Experimental Model. Pakistan BioMedical Journal, 6(3). https://doi.org/10.54393/pbmj.v6i3.897

Corresponding Author:
Muhammad Khalil Ahmad Khan
Department of Zoology, University of Okara, Renalla Khurd, Pakistan
dr.khalil@uo.edu.pk

Received Date: 6th March, 2023
Acceptance Date: 28th March, 2023
Published Date: 31st March, 2023

ABSTRACT

Carbon tetrachloride (CCl_4) is largely used as a solvent in chemical industries. It is also well known for hepatic and renal toxic actions. It imposes serious health threats. It is also one of the major causes that is toxic for the vital organs like lungs, kidney, liver, brain, etc. **Objective:** To check nephrotoxicity of Carbon Tetrachloride (CCl_4) on Rat Kidneys. **Methods:** The experiment was conducted at the animal house of the Department of Zoology, University of Okara. The targeted animal was Albino Rat. Two groups were designed control and experimental groups. The rats were fed with 30% diluted CCl_4 to check the toxic effect on the kidneys and normal saline to the control group for comparison. A trial for 12 days was conducted for this purpose. Sampling or dissection was done after 12 days to determine serum Urea, Creatinine, and Electrolytes Sodium (Na), and Potassium (K). Rats were dissected and the heart was punctured to take a blood sample and to collect organs. **Results:** We observed the increased values of Urea, Creatinine and Electrolytes, Sodium (Na), and Potassium (K) as compared to normal values, which have proved the renal toxicity was induced by CCl_4 in Albino Rats. All the experimental data were analyzed by using SPSS-19. The level of significance among the various treatments was determined by LSD at a 0.05% level of probability. **Conclusions:** These findings underline the substantial health risks that CCl_4 poses and emphasize the necessity of putting preventative measures and safety regulations in place.

INTRODUCTION

The molecular weight of carbon tetrachloride (CCl_4), which has four Cl atoms surrounding the carbon atom at its center, is 153.8 g/mol [1]. It is a colorless, transparent, volatile liquid. Free radicals generate lipid peroxidation and are thought to be one of the main factors in cell membrane deterioration, which can result in acute and long-term renal injury and a number of clinical conditions [2-5]. Furthermore, research on numerous proven case studies demonstrated that CCl_4 causes renal disorders in people [4]. According to earlier observations, CCl_4 produced oxidative stress in the kidney and lung as well as alterations in the mixed-function oxidases and microsomal cytochromes in the lungs of rats [6]. Human toxicity is typically brought on accidently by ingestion, dermal absorption via direct skin contact, or inhalation of its vapors; it can also be intentionally consumed as a suicidal agent. The liver, kidneys, and lungs are the primary organs in which CCl_4 damages cells [7]. Tetrachloride has been found to mostly harm the liver (swollen, painful liver, alterations in enzyme levels, and jaundice) and kidneys (nephritis, nephrosis, proteinuria) of humans during acute inhalation and oral exposures to high levels of carbon. There have also been reports of central nervous system depression [8]. Even at relatively large dosages, renal damage is rarely seen in animals exposed to carbon tetrachloride. Although the cause of animals' lower sensitivity to renal damage than humans is unknown, it may be related to how differently CCl_4 is metabolized by their kidneys. After 5 weeks of intermittent exposure to an anesthetizing dosage of carbon tetrachloride, the content of vitamin A in the kidneys doubled, and a 10% rise in wet organ weight was noted [9]. However, the concurrently

DOI: https://doi.org/10.54393/pbmj.v6i3.897

caused hepatotoxicity may have overshadowed this vitamin A effect. Rats subjected to 50 ppm for 5–10.5 months and monkeys exposed to 200 ppm for 10.5 months both showed a little renal edema. At an exposure level of 200 ppm, renal tubular degeneration became visible [10]. Through the production of free radicals, it has been noted that carbon tetrachloride (CCl_4) induces renal damage in rats [11, 12] in addition to hepatic toxicity [13]. Another study found that the protein content of renal tissues significantly increased after treatment with CCl_4. When CCl_4 was administered, proteins suffered oxidative damage and accumulated as a result of inadequate proteasomal and lysosomal breakdown, leading to metabolic inefficiency in the kidneys [14]. Similar to earlier investigations, the treatment of rats with the same substance reversed the shifts toward the control rats [12]. According to a histological analysis, CCl_4 treatment caused lipid peroxidation of the lipid structures in the renal tissues, which led to subcellular damages. The CCl_4-induced vasoconstriction results in an ischemia local environment, and SA has the effect of reducing the morphological changes that CCl_4 causes. Similar histological alterations were seen in renal CCl_4-treated rats, and these alterations resulted in a number of cellular impairments, including a decline in membrane integrity. The fact that the severe alterations were not seen in the groups given the same treatment suggests that rats given CCl_4 plus *Launaea procumbens* extracts had lost their protective effects. In other research [11, 12], similar histological observations were described. Free radicals generate lipid peroxidation and are thought to be one of the main factors in cell membrane deterioration, which can result in acute and long-term renal injury and a number of clinical conditions [3-5, 15]. In addition, findings on several case studies with documentation demonstrated that CCl_4 causes renal problems in people [4]. Both the kidney's inner medullary area and outer cortex have noticeable histological alterations. *Digera muricata* extracts appeared to have protective benefits in reducing the morphological alterations caused by CCl_4 in the groups that were not subjected to the severe modifications. Ogeturk *et al.*, (2005) found similar histological abnormalities in the kidneys of CCl_4-treated rats; however, these changes vanished in rats treated with CCl_4 + caffeic acid phenyl ester (CAPE) [3, 11]. In the kidneys of rats given CCl_4, tubular epithelial cell changes, such as vacuolization, atrophy, and ultimately epithelial cell detachment, suggested tubular necrosis. After prolonged exposure to CCl_4, similar histological changes were also discovered in other studies [16]. The capacity of tubular absorption may have been altered as a result of these histological changes, leading to the functional overload of nephrons and eventual renal dysfunction [5].

METHODS

In this investigation, adult male Albino Wistar rats weighing 180-200g were employed. The animals were bought from a nearby market and housed at the zoo's animal house at the University of Okara (Pakistan). Throughout the trial, they were kept in cages with four or five rats each, at a temperature of 25 3 °C, with 12-hour light/dark cycles and a minimum relative humidity of 44%. Rat Chow (20% crude proteins) and water were available at all times. Every dose was administered in the morning (Table 1). The animals were kept in these facilities for at least one week before the experiment.

Table 1: List of Groups, Doses, Days and Amount of Doze

Groups	Doses	Days	Amount
Group 1	Normal Saline	12 Days	1ml/kg
Group 2	30 % CCl4	12 Days	1ml/kg

Carbon Tetrachloride (CCl_4) was used for this study. The tested substance, carbon tetrachloride (CCl4), was bought from a nearby market and stored in the zoology department's lab at the University of Okara in Pakistan. The chemical is diluted by 30%. distilled water was used to produce the stock solutions. Freshly manufactured stock solutions were used for all of the working solutions. The other substances that were used were all of pro-analysis quality and came from conventional commercial sources. The number of rats were randomly assigned into two groups, a control group (Co) having fifteen (15) rats and an experimental group having the same number of rats as in the control group with exposure of 30% CCl_4 mixed with normal saline through oral gavage for 12 days. The experimental rats were anesthetized by putting in a desiccator exposed to chloroform and a small incision was made to cut the abdominal wall with sharp scissors. Then the muscular layer was cut on the sides to expose the internal organs. 0.9% pyrogen-free sodium saline solution was poured on the exposed organs of the animal to avoid drying. The dissections were done in completely aseptic conditions and tissues (heart, kidneys, liver, spleen & intestine) were excised. Normal feed was given to rats for 24 before dissection. Rats were euthanized with chloroform. Dissect the rats and by cardiac puncture, blood samples were collected in vacutainers by using 23 G1 syringes. Kidneys were dissected out, washed with ice-cold saline to remove debris. Organs were weighed and store at -20 ºC for tests. Centrifuge the blood samples at 10,000rpm for 15 minutes at 4 ºC. The serum was separated and store at -20 ºC. Urea, creatinine, and electrolytes (Na and K) in serum only write your parameters were estimated by using standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Austria). For the analysis of serum samples

DOI: https://doi.org/10.54393/pbmj.v6i3.897

of rats, the diagnostic kits were used to estimate urea, creatinine, and electrolytes levels in serum samples. Creatinine was determined using the Bartels & Böhmer (1971) technique [17]. The sample's creatinine combines with picrates in an alkaline solution to create a colorful complex at a wavelength of 500 nm. The amount of urea in the sample was determined using the Tabacco et al., (1979) method, which produced a colored complex that could be quantified by spectrophotometry (LKB, Sweden) at 600 nm [18]. Biochemical parameters measured were sodium (Na), potassium (K) using standard kits (Eve's Inn Diagnostics, Vadodara, India). Take blood in clot vile as a sample. Let it clot put it on the incubator for 5 to 6 minutes. Let it clot then centrifuge it 2,3 mint and then red blood cells came to the bottom and serum float over the cells. Then take the serum to test RTF (Urea, Creatinine, and BUN) and Serum Electrolyte. Before performing any test, we have to give washings to the machine to clean it. Apparatus that was used during the serum sample tests are Micro – Lab 300, Reagent Hemans or Diesis Reagent 1 and Reagent 2, Yellow and Blue Tip, Pipette 100ml and 1000ml Take Glass Tube, before using the tip and glass tube clean it well with tissue paper. Reagent 1and Reagent 2 were used 400ml and 100ml respectively by using the yellow tip. The total was 500ml. 5 ml serum was taken and after shaking it well, was read in Micro-Lab 300 by setting it at Chapter Urea. After two or three minutes, trial results appeared on the screen of Micro-Lab. The sample was taken in Clot Vile. Incubated it by incubator for 5 to 6 minutes to clot the blood then centrifuge it for 2 to 3 minutes. After centrifugation, plasma and blood cells were separated. Micro – Lab 300, Reagent Hemans or Diesis Reagent 1 and Reagent 2, yellow and blue tips were used, Pipette 100 ml and 1000ml were used. Take Glass Tube. Before using tips and glass tubes cleaned it well with tissue paper. After taking the sample in a clot vile, incubated it to clot the sample and then centrifuged it. Sodium reagent was taken in the amount of 1000ml and a 1000ml pipette was used. Incubate it at 35°C for 7 minutes. The sodium chapter was set at Micro-Lab 300. Samples were read through the machine and trial results were appeared on the screen. Samples were taken in clot vile. 500ml reagent was taken in the glass tube and 10-µl plasma was used. Incubated for three minutes and the potassium chapter was set at Micro-Lab 300. Results appeared on the screen. The computer program SPSS - 19 conducted a one-way analysis of variance to evaluate the treatment effects. LSD was used to calculate the significance level for each of the treatments at a level of probability of 0.05%.

RESULTS

As we know Carbon Tetrachloride (CCl_4) is a toxic compound and this toxic compound also exerts toxicity on the kidney. The analytical analysis showed that CCl_4 exposure alternates the normal physiology disturbing the normal values of urea, creatinine, Na, and K in the blood of the treated groups. Significant differences were observed in the hematology parameters between the control and treatment groups. We have found that those individuals who were exposed to CCl4 Urea levels are increased in them. Serum urea level was increased in treated groups (44.60 ± 1.68) in comparison to the control group by the value of (22.60 ± 0.95). Abnormally increased level of Creatinine was observed in CCl4 administered rats by the value of (1.386 ± 0.094) in comparison to normal control by the value of (0.88 ± 0.03) indicating the increased toxicity in rat's kidneys. An abnormally increased level of Sodium (Na) was observed in CCl4 administered rats in treated groups (139.20 ±.685) in comparison to normal control by the value of (136 ± 0.392) indicating the increased toxicity in rat's kidneys. An abnormally increased level of Potassium (K) was observed in CCl4 administered rats (4.722 ± 0.578) in comparison to normal control by the value of (3.68 ± 0.392) indicating the increased toxicity in rat's kidneys (Table 2 and Figure 1).

Table 2: Mean comparison between healthy and diseased group

	Healthy (n=15)	Diseased (n=15)	p-value
Total Urea	22.60 ± 0.95	44.60 ± 1.68	0.00
Creatinine	0.88 ± 0.03	1.386 ± 0.094	0.00
Na	136 ± 0.392	139.20 ±.685	0.00
K	3.68 ± 0.392	4.722 ± 0.578	0.00

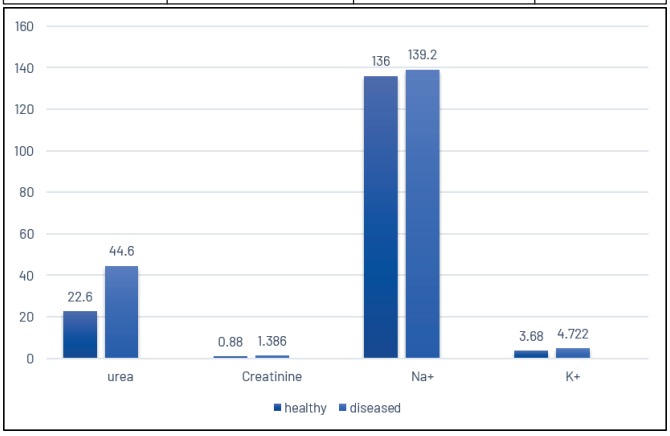

Figure 1: Mean comparison of the diseased urea, creatinine, Na^+, and K^+ with healthy group

One sample t-test was applied to determine the confidence interval of mean difference of Urea, Creatinine, Na, and K as shown in Table 3.

Table 3: T-Test Analysis

	N	Mean ± SD	Std. Error Mean
Urea	15	44.60±6.50	1.67843
Creatinine	15	1.38±0.36	.09411
Na	15	139.20±2.65	.68452
K	15	4.72±0.22	.05778

Table 4 shows confidence interval of the mean difference

DOI: https://doi.org/10.54393/pbmj.v6i3.897

of urea, creatinine, Na, and K. Upper and lower confidence interval for urea was 41.00 and 48.19 respectively. Upper and lower confidence interval for creatinine was 1.18 and 1.58 respectively. Upper and lower confidence interval for Na was 137.73 and 140.66 respectively. Upper and lower confidence interval for K was 4.59 and 4.84 respectively.

Table 4: Mean difference of urea, creatinine, Na, and K

Test Value = 0						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Total Urea	26.572	14	.000	44.60000	41.0001	48.1999
Creatinine	14.728	14	.000	1.38600	1.1842	1.5878
Na	203.353	14	.000	139.20000	137.7318	140.6682
K	81.727	14	.000	4.72200	4.5981	4.8459

Descriptive statistics were done of the treatment group at the significant variation of ($p > 0.05$) to determine the mean and standard deviation of urea, creatinine, Na, and K (Table 5).

Table 5: Descriptive statistics of the urea, creatinine, Na, and K

	N	Minimum	Maximum	Mean	Std. Deviation
Urea	15	38.00	55.00	44.6000	6.50055
Creatinine	15	.93	2.00	1.3860	.364472
Na	15	136.00	143.00	139.2000	.65115
K	15	4.40	5.01	4.7220	.22377

The (-.578*) value shows the association between urea and K, similarly the (.613*) indicates the correlation among the Na and K ions (Table 6).

Table 6: The correlation between the variables was determined by Pearson statistical analysis, at the significance level of 0.05

		Urea	Creatinine	Na	K
Urea	Pearson Correlation	1	-.211	-.492	-.578*
	Sig. (2-tailed)	-	.449	.062	.024
	N	15	15	15	15
Creatinine	Pearson Correlation	-.211	1	-.338	-.379
	Sig. (2-tailed)	.449	-	.217	.164
	N	15	15	15	15
Na	Pearson Correlation	-.492	-.338	1	.613*
	Sig. (2-tailed)	.062	.217	-	.015
	N	15	15	15	15
K	Pearson Correlation	-.578*	-.379	.613*	1
	Sig. (2-tailed)	.024	.164	.015	-
	N	15	15	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

This study was conducted to assess the toxicity of carbon tetrachloride (CCl_4) on albino rat kidneys. 30% diluted CCl_4 mixed with regular saline was fed to rats as food. The values of urea, creatinine, and the electrolytes sodium (Na) and potassium (K) were examined during this study. The findings demonstrated that the parameters in CCl_4-treated rats were considerably greater than those in control rats. When CCl_4 is consumed, the body's normal balance is disrupted, which results in kidney damage in albino rats. Similar findings were noted by Ogeturk *et al.*, Ozturk *et al.*, Simerville and Bhattacharya and Lun, (2005) [3, 16, 19, 20]. They looked at high levels of urea and creatinine in the urine as the key signs of renal damage brought on by CCl_4 treatment. In their experiment, they discovered that the serum creatinine level doesn't increase until at least half of the kidney nephrons had been compromised or lost. Both Adewole *et al.*, and Bhadauria *et al.*, noted comparable outcomes. They claim that the CCl_4-treated rats had significantly higher serum urea, creatinine, and BUN values, which is associated with decreased creatinine clearance [5, 21]. Similar findings and observations were made by Khan *et al.*, and Xu *et al.*, (2010) [11, 22]. They claimed that clinical chemistry data demonstrated that CCl_4 caused substantial increases in serum BUN and creatinine, which were consistent with published results and suggested potential renal damage. Eden *et al.*, noted comparable outcomes. They claimed that the elevated levels of renal creatinine caused by CCl_4 indicated that the chemical was responsible for several impairments of renal functions, including increased energy expenditure and reduced renal utilization of freshly synthesized creatinine [23]. Huxtable recorded comparable outcomes. He claimed that CCl_4 exposure significantly raised the levels of urine, electrolytes (Na & K), and creatinine in the rat kidney [24]. We saw higher amounts of each of these factors in this investigation. Adewole *et al.*, noted comparable outcomes. They claimed that chronic injection of CCl_4 resulted in considerable kidney oxidative stress and marked impairment of renal functioning [5]. With reduced creatinine and BUN clearance, serum creatinine and blood urea nitrogen (BUN) concentrations were considerably increased in CCl_4-treated rats. Melatonin (MEL) lowered the high levels of serum creatinine and BUN and greatly increased the clearance of creatinine and BUN. The capacity for tubular absorption may have been altered, leading to a functional overload of nephrons and associated renal dysfunctions, according to theory. Similar results were noted by Vengal Rao *et al.*, They claim that giving CCl_4 to normal rats caused kidney toxicity because it increased serum levels of creatinine and uric acid, which are indicators of the risk of impaired renal function and gout, respectively [25].

CONCLUSIONS

These findings underline the substantial health risks that CCl_4 poses and emphasize the necessity of putting preventative measures and safety regulations in place.

Authors Contribution

Conceptualization: MFB
Methodology: MKAK

DOI: https://doi.org/10.54393/pbmj.v6i3.897

Formal analysis: MFB
Writing-review and editing: MFB, MKAK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Unsal V, Cicek M, Sabancilar İ. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Reviews on Environmental Health. 2021 Jun; 36(2): 279-95. doi: 10.1515/reveh-2020-0048.

[2] Satyanarayana PS, Singh D, Chopra K. Quercetin, a bioflavonoid, protects against oxidative stress-related renal dysfunction by cyclosporine in rats. Methods and Findings in Experimental and Clinical Pharmacology. 2001 May; 23(4): 175-81. doi: 10.1358/mf.2001.23.4.634641.

[3] Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. Journal of Ethnopharmacology. 2005 Feb; 97(2): 273-80. doi: 10.1016/j.jep.2004.11.019.

[4] Manna P, Sinha M, Sil PC. Aqueous extract of Terminalia arjuna prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complementary and Alternative Medicine. 2006 Dec; 6 :1-10. doi: 10.1186/1472-6882-6-33.

[5] Adewole S, Salako A, Doherty O, Naicker T. Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. African Journal of Biomedical Research. 2007 May; 10(2): 153-164. doi: 10.4314/ajbr.v10i2.50619.

[6] Ganie SA, Haq E, Masood A, Zargar MA. Amelioration of carbon tetrachloride induced oxidative stress in kidney and lung tissues by ethanolic rhizome extract of Podophyllum hexandrum in Wistar rats. Journal of Medicinal Plants Research. 2010 Aug; 4(16): 1673-7.

[7] Teschke R. Liver injury by carbon tetrachloride intoxication in 16 patients treated with forced ventilation to accelerate toxin removal via the lungs: A clinical report. Toxics. 2018 Apr; 6(2): 25. doi: 10.3390/toxics6020025.

[8] Faroon O. Toxicological profile for carbon tetrachloride. Agency for Toxic Substances and Disease Registry; 2005.

[9] Chapman K, Prabhudesai M, Erdman Jr JW. Effects of ethanol and carbon tetrachloride upon vitamin A status of rats. Alcoholism: Clinical and Experimental Research. 1992 Aug; 16(4): 764-8. doi: 10.1111/j.1530-0277.1992.tb00675.x.

[10] Adams EM, Spencer HC, Rowe VK, McCollister DD, Irish DD. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. AMA Archives of Industrial Hygiene and Occupational Medicine. 1952; 6(1): 50-66.

[11] Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. Journal of Ethnopharmacology. 2009 Feb; 122(1): 91-9. doi: 10.1016/j.jep.2008.12.006.

[12] Khan RA, Khan MR, Sahreen S, Bokhari J. Prevention of CCl_4-induced nephrotoxicity with Sonchus asper in rat. Food and Chemical Toxicology. 2010 Aug; 48(8-9): 2469-76. doi: 10.1016/j.fct.2010.06.016.

[13] Lin HM, Tseng HC, Wang CJ, Lin JJ, Lo CW, Chou FP. Hepatoprotective effects of Solanum nigrum Linn extract against CCl_4-iduced oxidative damage in rats. Chemico-Biological Interactions. 2008 Feb; 171(3): 283-93. doi: 10.1016/j.cbi.2007.08.008.

[14] Srinivasan M, Rukkumani R, Ram Sudheer A, Menon VP. Ferulic acid, a natural protector against carbon tetrachloride-induced toxicity. Fundamental & Clinical Pharmacology. 2005 Aug; 19(4): 491-6. doi: 10.1111/j.1472-8206.2005.00332.x.

[15] Anjaneyulu M, Tirkey N, Chopra K. Attenuation of cyclosporine-induced renal dysfunction by catechin: possible antioxidant mechanism. Renal Failure. 2003 Jan; 25(5): 691-707. doi: 10.1081/JDI-120024285.

[16] Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology. 2003 Aug; 62(2): 353-6. doi: 10.1016/S0090-4295(03)00255-3.

[17] Bartels H and Bohmer M. Microestimation of creatinine. Clinica Chimica Acta. 1971; 32: 81-5. doi: 10.1016/0009-8981(71)90467-0.

[18] Tabacco A, Meiattini F, Moda E, Tarli P. Simplified enzymic/colorimetric serum urea nitrogen determination. Clinical Chemistry. 1979 Feb; 25(2): 336-7. doi: 10.1093/clinchem/25.2.0336a.

[19] Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. American Family Physician. 2005 Mar; 71(6): 1153-62.

[20] Bhattacharya H and Lun L. Biochemical effects to toxicity of CCl 4 on rosy barbs (Puntius conchonius). Our Nature. 1970 Jan; 3(1): 20-5. doi: 10.3126/on.v3i1.330.

[21] Bhadauria M, Nirala SK, Shukla S. Multiple treatment of propolis extract ameliorates carbon tetrachloride

DOI: https://doi.org/10.54393/pbmj.v6i3.897

induced liver injury in rats. Food and Chemical Toxicology. 2008 Aug; 46(8): 2703-12. doi: 10.1016/j.fct.2008.04.025.

[22] Xu JY, Su YY, Cheng JS, Li SX, Liu R, Li WX, *et al*. Protective effects of fullerenol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. Carbon. 2010 Apr; 48(5): 1388-96. doi: 10.1016/j.carbon.2009.12.029.

[23] Eden E, Harrison DD. Studies in Carbon Tetrachloride Poisoning. Australian Journal of Experimental Biology & Medical Science. 1955 Feb; 33(1): 85-89. doi: 10.1038/icb.1955.9.

[24] Huxtable RJ. Physiological actions of taurine. Physiological Reviews. 1992 Jan; 72(1): 101-63. doi: 10.1152/physrev.1992.72.1.101.

[25] Vengal Rao P, Ashok Kumar CK, Ashwini G, Ambareesh Kumar R, Sangeetha S. Evaluation of Hepatoprotective Activity of Santallum Album (Stem) Against Paracetamol Induced Hepatotoxicity in Albino Wistar Rats. International Journal of Innovative Pharmaceutical Research. 2014; 5(1): 370-373.