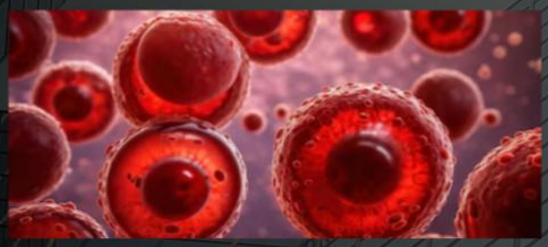
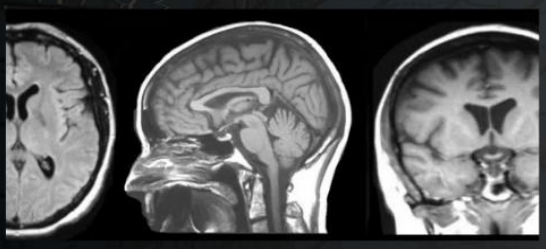
LIAQUAT MEDICAL RESEARCH JOURNAL

Official Journal of Diagnostic & Research Laboratory,
Liaquat University of Medical & Health Sciences, Jamshoro
Pakistan

ISSN-p:2664-5734
ISSN-o: 2709-5878





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VOLUME 7 ISSUE 2
1 April 2025 - 30 June 2025

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Liaquat Medical Research Journal is the print, online, double blind, peer-reviewed, quarterly released journal devoted to publishing innovative biomedical research and scholastic / academic content from all fields of medical sciences, concentrating on innovative clinical, diagnostic and perspective preventive research.

Aims & Scope

The Journal aims to publish research in all fields of clinical, diagnostic, experimental & preventive areas related to medical sciences to disseminate scholastic work among clinicians and scientists around the globe.

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Liaquat Medical Research Journal (LMRJ)
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Editorial

BENCH TO BEDSIDE: CHALLENGES AND OPPORTUNITIES IN LOW- AND MIDDLE-INCOME COUNTRIES

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DOI: 10.38106/LMRJ.2025.7.2-01
Received: 16.05.2025
Accepted: 10.06.2025
Published: 30.06.2025

ABSTRACT:
Translational research is a modern era of medical and clinical research where basic research discoveries are brought in the clinical practice to the patient care. The medical science has been revolutionized by these discoveries and clinical outcome of diseases has improved with longer survival and better quality of life. However, low-middle income countries (LMICs) are not enjoying the magic of translational research in its true sense as they are still dependent on discoveries from high income countries. There is no lacking of talent or ideas in LMICs but there are challenges like inappropriate infrastructure, shortage of trained human resource and also there are issues of funding for translational research projects. The medical research is expensive in fact but LMICs must see it as an investment. While working on local problems and finding their solutions locally, will certainly establish local research based business and the products can reach to the patient at low cost. Thus this may be taken as opportunity.

Keywords: Translational research, low-middle income countries, medical discoveries

INTRODUCTION

Translating laboratory research into clinical practice is defined as *Translational research*, which is basically a journey of a scientific theory from basic sciences laboratory i.e. Bench to the use in clinical practice to cure patients i.e. Bedside. It is a major milestone in modern medicine and recent research paradigm shift. The ability to convert molecular insights, genetic discoveries, and novel therapeutics into meaningful improvements in patient outcomes has revolutionized healthcare around the globe. However, major discoveries have come from high-income countries. While in low- and middle-income countries (LMICs), this journey remains fraught with obstacles, raising critical questions about equity, feasibility, and sustainability in medical innovation.

The Paradox of Discovery and Delivery

The LMICs have talent, ideas and capability of innovation. The science in these regions is still facing challenges like infectious diseases even at advance level, in addition, the burden of non-infectious diseases is also there same as high-income countries. Researchers in these regions do involve in innovative research in their laboratories but these discoveries seldom reach to the patient care or pharmaceutical market, not even within their own country. It is an important aspect to realize that there are certain local issues, including health issues such as predominance of particular diseases, thus local research is always there, but the issues related to the access of the infrastructure to test these discoveries, and integrate these innovations into health systems remain limited.

Barriers to Translation of basic research into bedside clinical practice

There are several barriers involved:

1. **Infrastructure Gaps**: Clinical trial facilities, advanced diagnostic platforms, and biobanks are scarce, limiting the capacity to conduct translational studies.
2. **Funding Limitations**: Research budgets are often minimal and heavily reliant on international donors. This dependence means priorities are frequently externally set and may not align with national health needs.
3. **Regulatory Bottlenecks**: Lengthy, under-resourced, or fragmented ethical and regulatory processes delay trials and discourage collaboration with industry. The registration and approval of the sites are also expensive in terms of infrastructure and human resource, thus further limit the testing of the discoveries.
4. **Human Resource Constraints**: While there is no shortage of motivated clinicians and scientists, few are trained in translational research, and even fewer receive mentorship in cross-disciplinary collaboration.

5. **Weak Academia-Industry Linkages**: In many LMICs, partnerships between universities, research institutes, and local pharmaceutical or biotech industries are either underdeveloped or non-existent.

Patient as partner in translational research

The involvement of patients is equally important as testing in a clinical scenario make the route of the discovery to the market. Patient involvement starts from Phase I to Phase IV. From patients point of view, they often present late in the disease course, face financial barriers to care, and are excluded from advanced therapies developed elsewhere, and understandably cannot be included in initial stage of the disease. Literacy rate of LMICs is also low thus compliance of the trial/ research protocol is often a question is be taken care at the time of study designs.

Opportunities amid Challenges

Despite all these barriers there are possibilities of creating opportunities, there are a few mentioned here:

1. **South-South Collaborations**: Increasing partnerships between LMICs allow resource-sharing, pooled expertise, and regional clinical trial networks. In addition, researchers from limited-resource countries may understand each other in making situational decisions.
2. **Digital Health Solutions**: Mobile health, telemedicine, and AI-based decision support tools can accelerate translation by bypassing some infrastructure gaps.
3. **Policy Reforms**: Some countries with low resources have demonstrated that how supportive policies, investment in local biotech, and regulatory streamlining can foster translational ecosystems.
4. **Capacity Building**: Training clinician-scientists in translational medicine and embedding research within clinical practice can bridge the current divide.

For LMICs, the challenge is not only scientific but systemic. Governments, academia, industry, and civil society must recognize translational research as a national priority and view it as a business opportunity for local economic growth. Local health problems demand local solutions, and these solutions must reach patients without delay and also at an affordable cost. International collaborations should shift from extractive models to equitable partnerships, ensuring that LMIC populations benefit from discoveries to which they contribute.

CONCLUSION

The journey from bench to bedside in LMICs is long and uneven, but it is not insurmountable. By strengthening infrastructure, investing in human capital, and fostering collaborations rooted in equity, LMICs can carve pathways for research that is not only innovative but also transformative for the patients who need it the most. Translational research, when contextualized to the realities of resource-limited settings, has the potential to redefine healthcare delivery and bring science closer to those it seeks to serve.

Conflict of Interest

Author declare no conflict of interest.

Liaquat Medical Research Journal (LMRJ)
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Original Article

PREVALENCE AND AWARENESS OF OVER-THE-COUNTER MEDICATION: ADDRESSING THE ALARMING SURGE OF SELF-MEDICATION IN YOUNG POPULATION

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DOI: 10.38106/LMRJ.2025.7.2-02
Received: 23.04.2025
Accepted: 15.06.2025
Published: 30.06.2025

ABSTRACT:

Globally, over-the-counter (OTC) drugs are commonly used as nonprescription medications. Among these, analgesic agents are most frequently consumed, though their indiscriminate use can lead to serious health complications. The use of OTC medicines without prescription and proper awareness has now become a public health concern world-wide. The objective of this study was to assess the awareness, prevalence and handling of OTC medicines among the students of a local university. A cross-sectional survey based study was conducted using a structured open-ended questionnaire, distributed randomly among university students in Karachi, Pakistan. A total of 300 students participated in the study. The questionnaire aimed to evaluate participants' knowledge and usage patterns of common analgesics such as paracetamol, ibuprofen, and aspirin. Out of 300 participants 69% were females and 31% were males, aged between 18 to 30 years. Among them, 56% reported that paracetamol, ibuprofen, and aspirin were used for pain relief, while 44% were unaware of its indication. Pain was the most commonly reported reason for OTC drug use (29%). Awareness of adverse effects was also assessed, with gastrointestinal (GI) complications being the most reported side effect at 43%. The OTC are commonly reported to be taken for pain.

Keywords: Over the Counter (OTC) medications, awareness, adverse effects, cross-sectional study, analgesics

INTRODUCTION

Self-medication is defined as the *active management by individuals of their own treatment, symptoms, lifestyle, and physical or psychological consequences from acute or chronic conditions*(1). Self-medication is viewed as a potential solution to alleviate the growing strain on healthcare and social resources while lowering associated costs, with the assumption that effective self-care reduces the need for healthcare services (2-3). This approach has gained prominence in health policy discussions in the United Kingdom (4).

Pain, a common human experience, is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"(5). This definition acknowledges both the sensory and emotional aspects of pain. Pain is now recognized as the fifth vital sign in clinical assessments and is one of the most significant symptoms prompting individuals to seek medical attention (6).

The use of non-prescription drugs (NPDs), including over-the-counter (OTC) medications, is widespread globally (7). These medications are used to treat a variety of mild to moderate conditions such as pain, fever, and behavioural issues like irritability or restlessness (8). The OTC drugs are available to consumers without a prescription, in contrast to prescription-only medications that require a valid prescription from a healthcare provider (9). The accessibility of OTC medications enables individuals to treat many ailments independently, without the supervision of a healthcare professional (10).

While OTC drugs offer benefits like ease of access and self-management, they also pose risks such as misdiagnosis, overdose, and drug interactions (11). In many countries, certain analgesics are available OTC without a prescription. When used correctly and in recommended doses, OTC medications are considered safe and effective for the general population (12). Paracetamol (also known as acetaminophen) is one of the most widely used OTC analgesics, known for its mild analgesic and antipyretic properties. It is available in both prescription and non-prescription forms (13).

Other commonly used analgesics include non-steroidal anti-inflammatory drugs (NSAIDs), which are frequently self-administered for both acute and chronic pain (14). The NSAIDs, which inhibit cyclo-oxygenase (COX) enzymes, are used for a wide range of conditions, from short-term relief of common ailments like colds, flu, headaches, and muscular pain to long-term management of chronic inflammatory diseases like rheumatoid arthritis (15,16). Some NSAIDs, such as aspirin and ibuprofen, are available OTC in many countries, including the United States (17).

The NSAIDs are available in both prescription-only and OTC formulations, often with the same dosage but marketed under different names or packaging for different uses (18). Despite their widespread use due to their analgesic and anti-inflammatory properties, NSAIDs are associated with serious adverse drug events, particularly affecting the gastrointestinal, cardiovascular, and renal systems (19, 20).

To manage the risks associated with OTC medications, healthcare professionals and patients must be educated about safe usage. A report from the British Medical Association (BMA) has highlighted the increasing trend of switching more potent medications from prescription-only status to OTC availability. This shift underscores the importance of enhancing education and awareness to ensure the safe use of these drugs (21). Therefore, this study was conducted to extent of the use of OTC and awareness of the users.

METHODS:

This study was based on a survey based approach to determine the awareness, prevalence and general understanding of the participants regarding the use of OTC drugs and their adverse effects. The handling of OTC drugs and reasons for purchasing OTC drugs were also investigated in this survey. The study was conducted from January 2024 to June 2024. Minimum sample size of the study was 300 and sample size was calculated by precision analysis technique ($n = Z^2\ p\ (1\text{-}p)^2/d^2$).

The individuals who participated in the study were selected from various departments of a public university in Karachi, Pakistan. The majority of the participants were females compared to males. All the protocols were according to standard practices.

Data was collected by using a questionnaire which was designed to obtain demographic details and to investigate the awareness of the participants about OTC drugs and their adverse effects. The content of the questionnaire was reviewed by the researchers and minor revisions were made for accuracy of questionnaire. Participants were asked about demographic information such as age and gender. They were also inquired about the general OTC drugs they are using and the adverse effects of those drugs after using for a certain period of time. Several questions regarding the perception and handling of OTC drugs was also included in the questionnaire. The study included both male and female participants aged 18 years and above. Those individuals who were below 18 years and who decline to participate were excluded from the study.

Statistical analyses

Data were analysed using Statistical Package for Social Sciences (SPSS version 20.0 Armonk, NY: IBM Corp) and were subjected to descriptive analysis where non-parametric test, i.e. chi-square test were applied. All the values were considered significant when $P < 0.05$. The results were presented in the form of frequencies, graphs and tables to facilitate easy interpretation.

RESULTS:

During the study period, 300 individuals participated in the study, including 207 females and 93 males. The age range of respondents were between 18 to 20 years (38%), 21 to 25 years (41%) and 26 to 30years (20%) respectively. The demographic information is shown in Table 1. Analgesics were most frequently used which included paracetamol, ibuprofen and aspirin. The survey results revealed that 56% people were aware about the indications of these analgesics whereas, 44% were unaware (Figure 1). The results showed that females had statistically significantly higher more knowledge about analgesics as compare to male (Table 2). The consumption of some commonly used OTC drugs such as Paracetamol, ibuprofen and aspirin was also investigated. It was found that Paracetamol was most commonly used (23%) among different analgesics however the use of other analgesics was below or up to 10% i.e. ibuprofen (6%), aspirin (10%) for pain and some other analgesic drugs are consumed up to 5% as shown in figure 2. The consumption of OTC medications for various illnesses was also evaluated.

According to the survey result, 29% participants used analgesics for various pain illness. 24% uses analgesics to relieve headache. For muscular aches and fever in 22% and for joint pain in 5% participants (Figure 3). Excessive use of OTC medications was also reported to be associated with various adverse effects including gastrointestinal (GI) complications such as diarrhea, vomiting in 43%, stomach bleeding in 2% and Renal complications and kidney failure was 12% and 11% respectively. Some other complications was also found which was up to 10%. It was found that 56% youngsters were well aware about addiction and dependence caused by the analgesic agent where as 14% were not aware while 22% participants have no idea about the addiction as shown in figure 4. Table 4 displays the results of an investigation about perceptions of the safety of over-the-counter medications. In addition, a great majority of responders (i.e. 59.66%) failed to verify expiration dates. About 18% of respondents confirmed that they read medicine leaflets that were placed in drug packaging prior to utilizing over-the-counter medications. Table 5 represents several reasons for which the respondents' purchases of over-the-counter medications for self-medication. According to 71% of participants, visiting the hospital is a waste of time, and 53.33% think they can take care of themselves if there is minor illness. When asked how long they keep over-the-counter medications at home before throwing them away, 230 (76%) said they keep them for less than a year, while 53 (17%) say they keep them for more than a year.

Table 1. Demographic characteristics of the study population

Sociodemographic Features		Frequency	Percentage
Gender	Male	93	31%
	Female	207	69%
Age	18-20 years	114	38%
	21-25 years	123	41%
	26-30 years	61	20.3%
Marital Status	Married	77	25.6%
	Unmarried	223	74.3%
Qualification	Undergraduate	245	81.6%
	Post Graduate	55	18.3%

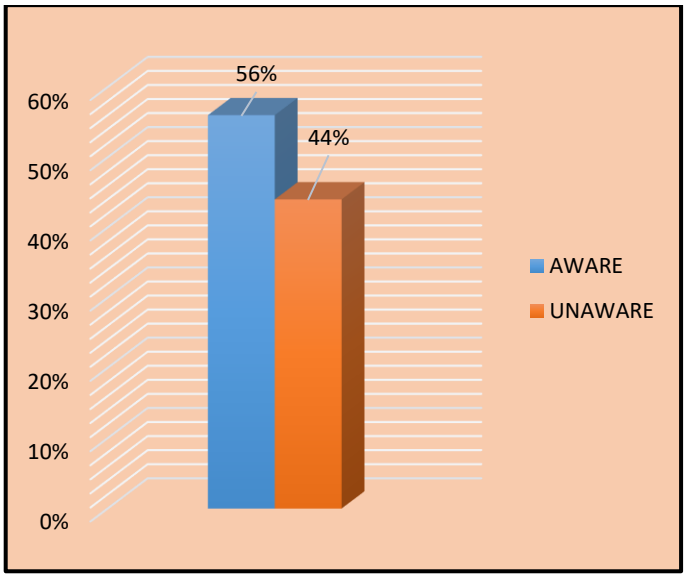

Figure 1. graphical presentation of responses

Table 2. Comparison of response of males and females

Gender	n (%)	n (%)	p-value
Male	19 (20)	74(80)	0.001
Female	157(76)	50(24)	
Total	176(58.6)	124(41.33)	

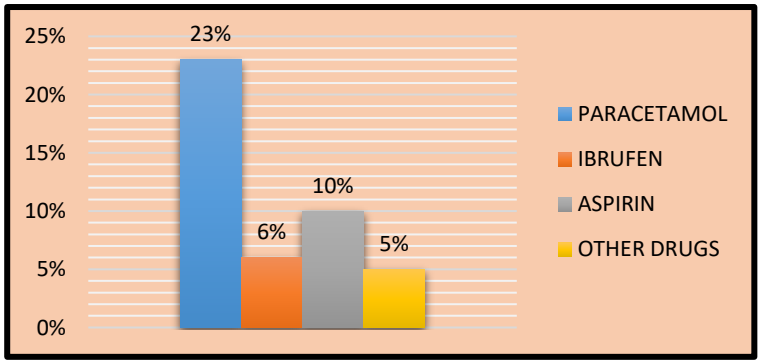

Figure 2 : Consumption of Different Analgesics by participants

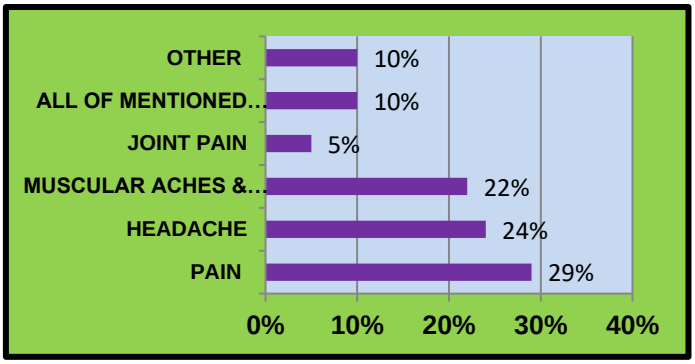

Figure 3: Different reported illnesses for which analgesics were used by participants

Table 3. Statistical analysis of adverse effects associated with analgesics

Adverse effects	Responses	Yes(%)	No (%)	Chi Square
GI* Complications	43	26	17	0.004
Renal Complications	12	8	4	
Kidney Failure	11	5	6	
Stomach bleeding	8	3	5	
Other complications	10	6	4	

*GI= Gastrointestinal complications

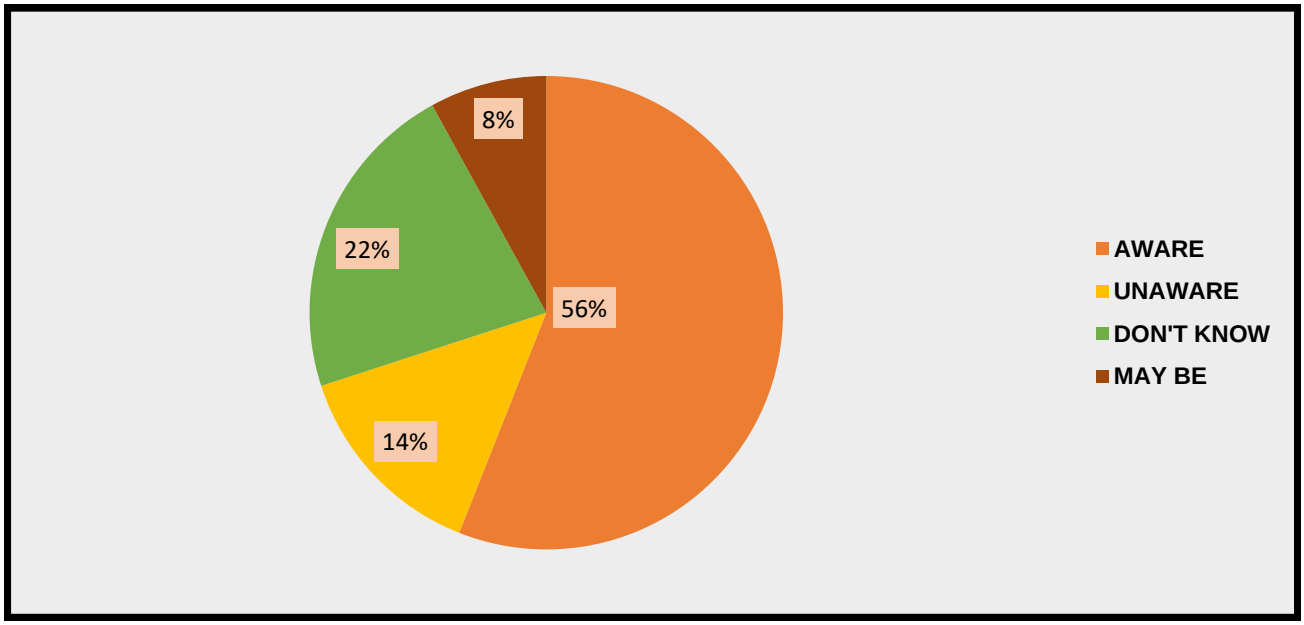

Figure 4. Awareness about addiction and dependency of Over The Counter (OTC) drugs.

DISCUSSION

According to the World Health Organization (WHO), nonprescription medicines are drugs that have been approved by health authorities for treating minor ailments and symptoms and available without prescription. These OTC medications are deemed safe and effective when used as directed, either according to the package instructions or on the advice of a pharmacist (22). The OTC medicine self-medication is a practice that poses a global public health concern. The prevalence of self-medication varies by target population and country (23, 24).

In this study we have examined OTC drug use patterns and assessed patients' perception about OTC drugs use in university students. The main finding of this study showed analgesics were the most commonly used as OTC drugs, where 56% of participants were aware about OTC medications, however, 44% participants were completely unaware. The most frequently used medications were paracetamol, ibuprofen and aspirin. Headache, fever and joint pain were the most frequent conditions for which OTC drugs were purchased. These findings are consistent with previously reported studies (25, 26).

When used properly, self-medication using over-the-counter medications can be advantageous as it can lessen various pain conditions and cut down treatment expenses and doctor visits (27). However, if used improperly, it can also pose a risk to human health and cause serious health issues. The participants reported that gastrointestinal issues, renal difficulties, kidney failure, stomach hemorrhage, and other complications were among the several complications caused by OTC drugs.

According to the participants, the most frequent reasons for using over-the-counter drugs were the low severity of the disease, easy access to the pharmacy, and time-consuming hospital visits. Numerous studies have documented various reasons why people self-medicate with over-the-counter drugs (28, 29). Regarding the expiration dates, majority of respondents stated that they do not verify the expiration date before using over-the-counter medications while others responded that they dispose them away if they observe a change in the medications' physical appearance. Just a small percentage of participants, however, stated that they see a pharmacist if over-the-counter medications fail to relieve a symptom or if the medication's color, smell, or form changes. These results suggest that community pharmacists need to adequately inform their patients about the expiry and physical changes of over-the-counter medications.

Despite the fact that the majority of respondents handled over-the-counter medications well, nearly all of them engaged in risky practices. Moreover, a few respondents stated that they do not read drug information leaflets prior to using over-the-counter medications. This research highlights the necessity of pharmacists counselling and educating consumers about OTC medications and the risks they pose if misused.

Community-based pharmaceutical counseling is especially important when people buy medications OTC without a doctor's prescription. Many customers in this survey believe that OTC medications are safe when used in excess of the recommended dosage, and the remaining respondents agree that OTC medications are generally safe regardless of their use. These findings suggest that participants most likely lack a thorough understanding of a number of risk factors associated with OTC medications, including those about drug-drug and drug-release interactions. According to previous studies, users tend to underestimate the hazards associated with OTC drugs and believe they are harmless (30, 31).

In general, patients have a positive perception of community pharmacists, though in certain situations, their understanding of pharmaceutical care services remains limited. Many patients are unaware of the risks associated with improper OTC medication use. Therefore, it is crucial for pharmacists to provide counselling at the time of purchase, ensuring the safe and effective use of these medications (32).

Since this study was cross sectional, the results of the study are dependent on the responses given by the study participants thus, there may possibility of respondent bias. The sample size was limited to university students and may not be representative of the general population.

CONCLUSION

The study revealed that a significant proportion of participants rely on non-prescribed medications rather than prescribed ones. It was found that paracetamol is considered as the safest and most effective first-line agent for pain relief, with 60% of participants choosing it. However, the routine use of nonprescription medications for various reasons could lead to complications and even addiction. The misuse of OTC drugs is alarmingly high, emphasizing the need for community pharmacists to promote proper use through counselling. Additionally, drug regulatory authorities should strictly enforce laws related to drug dispensing.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by local research ethics committee of University of Karachi and conducted according to the ethical principles and participants were informed about the purpose and scope of study. The consent was obtained from each respondent and the responses were kept confidential and anonymous.

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Original Article

ASSOCIATION OF THYROID HORMONE WITH LIPID PROFILE IN PATIENTS VISITING DISTRICT RAWALPINDI HOSPITAL, PAKISTAN

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DOI: 10.38106/LMRJ.2025.7.2-03
Received: 23.04.2025
Accepted: 15.06.2025
Published: 30.06.2025

ABSTRACT:

Current study aimed to examine the association of thyroid hormone with lipid profile in hospitalized patients. 5 ml venous blood sample was collected for evaluation of thyroid and lipid profiles. The study was conducted during January to June 2023 in Rawalpindi Institute of Cardiology (RIC), Rawalpindi, Pakistan. A total of 200 patients were recruited, among these 68 (34%) were females and 132 (66%) were males. On the basis of family history 64 (32%) patients had a family history of heart diseases, while 136 (68%) did not report any such history. The higher proportion of patients had hypothyroidism as compared to hyperthyroidism. Thyroid disorders along with lipid profile have greatly affected the cardiovascular system, consequently the rate of cardiac diseases has increased. The change in thyroid hormone levels can also affect lipid profile which will be directly affecting cardiovascular system even in low risk patients such as those without family history of cardiovascular diseases and without personal history of hypertension and diabetes mellitus. Thus it is essential to evaluate such relationship.

Keywords: Hypothyroidism, dyslipidemia, hyperthyroidism, triiodothyronine, lipid profile

INTRODUCTION

Thyroid hormones (TH) impact almost every organ function and have a broad influence on human body. The thyroid hormones play key role in regulating metabolism. Thyroid hormones affect blood pressure regulation, energy expenditure, and the metabolism of lipids and glucose in different ways (1). Over previous three decades, the incidence of metabolic syndrome has increased globally, rising from 1.1% in 1980 to 3.85% in 2015. Between 1990 and 2015, there was a 28.3% increase in the worldwide risk of mortality due to high body mass index (BMI)(2). Growth, development, and metabolism are all significantly regulated by TH, which is also crucial in controlling anabolism and catabolism of fats (3). The effects of hypothyroidism on blood lipid profiles are distinct. It has been reported that individuals with Thyroid Stimulating Hormone (TSH) >10 mLU/L have higher levels of ApoB-containing lipoprotein cholesterols as compared to the individuals with TSH 4.0–10.0 mLU/L (4, 5).

The levels of ApoB-containing lipoprotein cholesterols are always positively associated with the circulating TSH level, regardless of thyroid function (6,7). Accordingly, the likelihood of developing dyslipidemia increases with a higher TSH level (8, 9). Diabetes mellitus was also linked to a significantly higher risk of cardiovascular illnesses, especially type 2 diabetes, which was primarily linked to lipoid abnormalities. Notably, compared to the general population, diabetic patients had a higher frequency of thyroid dysfunctions (10). The present study was aimed to evaluate the association of thyroid hormone with lipid profile in hospitalized patients.

METHODS:

The study was conducted during a period of six month, from January 2023 to June 2023 in Rawalpindi Institute of Cardiology (RIC), Rawalpindi, Pakistan. For this study the individuals were selected who visited to the hospital with the complaint of cardiac origin with and without family history between the ages of 40 to 80 years. For all patient 5 ml venous blood sample was collected for evaluation of the thyroid and lipid profiles. The collected blood was allowed to clot by placing in a rack at room temperature for at least 30 minutes and maximum for 1 hour. Then it was centrifuged at 3,000 rpm for 5 minutes, and the separated serum sample was stored at -20 °C.

Biochemical analysis

The clear serum obtained from the blood was analyzed for TSH, and both thyroid hormones including Triiodothyronine (T3) and Thyroxine (T4) by using the chemiluminescent micro particle immunoassay (CMIA) technology. Lipid profile (cholesterol and triglyceride) were analyzed on Backman Coulter automated analyzers AU5800 and AU700 (Beckman Coulter, Inc., Crea, CA) by enzymatic photometry.

Statistical analyses

The Statistical Package for Social Sciences (SPSS software version 20.0) was used to analyze the collected data. A t-test was employed to compare the variables and a p- value <0.05 was considered statistically significant.

RESULTS:

A total of 200 patients were examined, among these 68 (34%) were females and 132 (66%) were males. On the basis of family history 64 (32%) patients reported to have positive family history of cardiac disease, while 136 (68%) denied any such history. The higher proportion of patients were found to have hypothyroidism as compared to the hyperthyroidism (Figure 1). In both males and females, the TSH (mean = 2.958) showed positive correlation between HDL-C (p=0.965, r=0.003), LDL-C (p=0.37, r=0.006), TG (p=0.16, r=0.095) and cholesterol (p=0.048,r=0.0597). In both the triiodothyronine (r=0.1490) there is positive correlation between cholesterol and positive correlation between HDL-C(r=0.1024), LDL-C(r=0.1205) and TG (r=0.2016). There was positive correlation between thyroxin (p= 0.14, r=0.1029) and HDL-C, TG (r=0.0475) and correlation between T4 and LDL-C is (r=0.05934) and cholesterol is (r=0.08468). Association between T3, HDL-C and TG considered statistically significant. Myocardial infraction had highest values with 20 (60.00%), while heart failure has lowest values with 1 (0.50%) respectively. During examination only 70 (35%) patients were smoker, while 130 (65%) were non-smoker (Table 1).

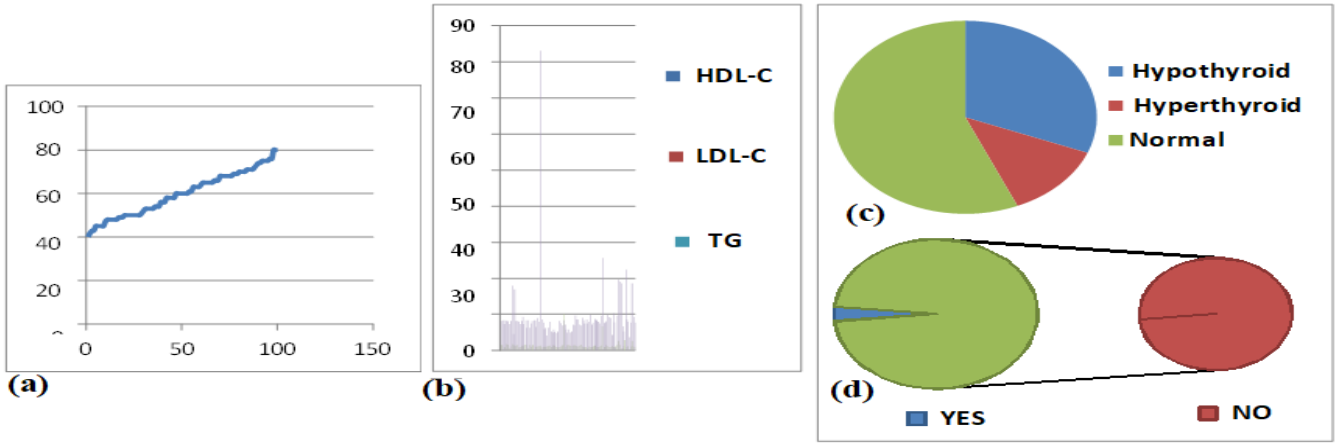

Figure 1. (a) Age of patients between 40 to 80 years; **(b)** Different values of lipid profile; **(c)** Number of patients with hypothyroid, hyperthyroid and normal; **(d)** Family history of dyslipidemia.

Table 1. Correlation of thyroid hormones with HDL-C, LDL-C, TG, cholesterol and number of patients with heart diseases.

Para	HDL-C *(p value)*	LDL-C *(p value)*	Triglyceride *(p value)*	cholesterol *(p value)*	% of heart disease in patients	
	(r)	(r)	(r)	(r)		
TSH	+0.0031 (0.9652)	-0.0627 (0.3777)	-0.0991 (0.497916)	-0.048 (0.497916)	Angioplasty 15 (7.50%)	Heart failure 1 (0.50%)
T4	+0.1343 (0.5796)	+0.0922 (0.1941)	+0.0434 (0.541)	+0.0251 (0.072424)	Arrhythmia 9 (4.50%)	Myocardial infraction 120 (60.00%)
T3	0.1024 (0.1490)	+0.1205 (0.8919)	+0.2017** (0.004*)	+0.0884 (0.213217)	Chest pain 13 (6.50%)	Unstable angina 11 (5.50%)
* p<0.05 statistically significant; ** p<0.01 statistically significant. Para: Parameters; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; TSH: Thyroid stimulating hormone; CAGB: Coronary artery bypass graft surgery.					CABG 7 (3.50%)	Suspected 24 (12.00%)
					% Smoker 70 (35%)	% non-smoker 130 (65%)

DISCUSSION

The level of thyroid hormones, especially hypothyroidism, the thyroid stimulating hormone level is significantly more as compared to normal subject. The locality and the environment are also affected the case profile of the patients as well as the controls. This hypotheroid state may be influenced by the altitude of the area of the samples. The study11 thyroid dysfunction was found in 22% of the 197 participants. Of these, 86 (35 females and 51 males) had a metabolic syndrome diagnosis, while the remaining 111 subjects (51 males and 35 females) were regarded as controls. In participants with metabolic syndrome, most of the hormone such as T3, and TSH were considerably higher ($p<0.001$) as related to without metabolic syndrome. Regarding the lipid profile, the individuals with metabolic syndrome had triglycerides of 262.8±112.3 mg/dL, which was substantially greater ($p<0.001$) than the triglycerides of those without metabolic syndrome (137.9±19.01 mg/dL). The group without metabolic syndrome had serum HDL levels that were considerably higher ($p<0.001$) at 50.5±3.9 mg/dL compared to 43.4±5.2 mg/dL in the metabolic syndrome group. Subjects with metabolic syndrome had TSH levels 5.3±3.4 µl/mL, which were substantially higher ($p<0.001$) than those without metabolic syndrome (2.6±1.4 µl/mL).

According to a study12 the median age for men was 39 (35–47) years, while the median age for women was 36 (32-43) years. 13% had hypothyroidism and 3% had hyperthyroidism; subclinical hypothyroidism accounted for 19% of thyroid dysfunction cases. Between hypothyroid and euthyroid patients, there were very significant variations in total cholesterol and thyrotrophin levels, with the former having more atherogenic profiles. When dyslipidemia was evaluated using logistic regression, a significant correlation with hypothyroidism was found (3.24(1.81-5.81), $p<0.001$). A total of 324 people were evaluated by13 the T4 was requested for each subject whose TSH was abnormal. Three categories were used to group the participants: euthyroid (226 individuals), subclinical hypothyroidism (75 individuals), and overt hypothyroidism (23 individuals). The lipid profile while fasting was assessed for cholesterol, TG, LDL, and HDL. Between research groups, there was a significant difference in LDL, TG, and cholesterol, but not in HDL (euthyroidism, subclinical, and overt hypothyroidism). Compared to euthyroidism, overt hypothyroidism demonstrated a substantial difference in LDL, TG, and cholesterol.

The study had a small sample size and only thyroid hormones and lipid profiles were assessed without taking potential confounding factors into account is taken as the limitations of the study. However, the study has provided evidence to explore thyroid hormone as one of the underlying cause for metabolic syndrome and cardiac abnormalities.

CONCLUSION

In conclusion thyroid disorders along with lipid profile has greatly affected the cardiovascular system, as a result heart diseases arise. Thyroid hormone plays important role in regulation of metabolism. So, with the increasing activity of thyroid hormone, marked changes in production of thyroid hormone occurs causing impact on the metabolism. In the thyroid patients when the level of thyroid hormone is altered then as result the dyslipidemia occurred. Dyslipidemia is metabolic irregularity in the thyroid patients and made the end result of the thyroid with the lipid metabolism that leads to the change in the cholesterol, triglycerides and the phospholipids. Further studies are required to establish string causal relationship.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by local research ethics committee.

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Liaquat Medical Research Journal (LMRJ)
Open access, Peer review Journal OPEN ACCESS

Original Article

ANTIMICROBIAL, ANTIOXIDENT AND CYTOTOXIC EVALUATION OF IFLOGA SPICATA (Forssk) Sch.Bip. BIOSYNTHESIZED SILVER NANOPARTICLES

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DOI: 10.38106/LMRJ.2025.7.2-04
Received: 04.10.2024
Accepted: 03.03.2025
Published: 30.06.2025

ABSTRACT:

The study aimed to determine pharmacological and phytochemical potential of different solvent fractions of Ifloga spicata. Antimicrobial potential, free radical scavenging activity 1, 1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide scavenging assay, and cytotoxic effects of plant samples on human blood lymphocytes under H_2O_2 induced stress was performed. Ifloga spicata ethyl acetate fraction markedly showed higher antibacterial potential against P. aeruginosa (ATCC 27853) and E.coli (ATCC 25922) bacterias. While higher antifungal potential was shown by the chloroform fraction against all three different strains A. fumigates (ATCC 204305), A. flavus (ATCC 9643) and A. niger (ATCC 16404) as compared to others fractions of the sample. DPPH maximum antioxidant potential was noted for methanolic extract followed by ethyl acetate with order of Methanol>Ethyl acetate>Chloroform>n-Hexane. Similarly, the hydrogen peroxide effect was significantly decreased by ethyl acetate fraction. In blood lymphocytes the raise in ROS and TBARS level due to stress given by H_2O_2 was also recovered by different extracts of I. spicata. The decrease level of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) was also restored to its normal by all extract of the Ifloga Spicata.

Keywords: Ifloga spicata, Antimicrobial, Reactive oxygen species, Human blood lymphocytes, Hydrogen peroxide.

INTRODUCTION

Herbal medicines have been practiced since ancient period and still raising in demand of the modern era, because multiple drugs resistance has been developed against microbial diseases due to irregular use of the synthetic drugs. In order to overcome the resistance side effects of the synthetic drugs scientists are trying to use herbal medicine against microbial infections. Medicinal plants contain certain bioactive ingredients which are used for various therapeutic purposes. In the developing countries approximately 60% to 90% peoples are using herbal plants for prevention of various diseases(1-2). The development of newly safe antimicrobial drug is one of the ever raising demands. Pathogenic bacteria have adverse effects on human and livestock all around world (3). Therefore, to minimize the adverse effect of these pathogenic microbes scientists are trying to identify and isolate antimicrobial agents of plant origin (4). Fruits, seeds, flower, leaves and stem possess various bioactive agents with antibacterial, phytochemicals and antioxidant potential (5-6). During metabolism Hydrogen peroxide is produced. In normal metabolism hydrogen peroxide (H_2O_2) is converted into water through peroxiredoxins, catalases and peroxidases and glutathione (7). But when this normal metabolism is disturbed then hydrogen peroxide (H_2O_2) directly form bond with transition metal and thus form hydroxyl radicals by Fenton reaction. These radicals then attack on DNA molecule sugar component and damages a single DNA molecule stand (8). To overcome the adverse effect of these agents medicinal plants are used as anticancer, anti-inflammatory agents and as management for digestive problems (9). Naturally there are different varieties of medicinal plants in Pakistan (10-11). Based on ethno medicinal value Ifloga spicata is used in present study. I. spicata belonging to family Asteraceae, is an annual herb having height of 5-10 cm usually branched at the base. The stems and branches of the plant make dense globular to cylindrical inflorescence either at base or in the upper portion. It usually prefers to grow in desserts. Ifloga spicata

is mostly distributed in Canary Island, southern Spain to N. Africa through Middle East to Afghanistan, Pakistan and India. Flowering period is February-September (12). These plants are readily available and have potential to be used as therapeutic options. However, there is limited literature available. Therefore, this study was aimed to determine antimicrobial, antioxidant and cytotoxic potential of I. spicata extracts on human blood lymphocytes exposed to H_2O_2 oxidative stress.

METHODS:

Plant Collection:

The plant I. spicata was collected from Bannu District Khyber Pakhtonkhwa, Pakistan. Dr. Faizan Ullah Khan Assistant professor Department of Botany University of Science and Technology Bannu identified the plant. It was assigned with a voucher No (Is-I5) and was stored in Deportment of Botany University of Science and Technology Bannu.

Extraction and final product preparation:

Plant material was first washed with tap water followed by distilled water. The plant material was dried under shadow condition, converted into fine powder. 5 Kg powder of I. spicata was dissolved in 80% aqueous methanol and was placed for several days. After complete dissolution, the methanolic solution of I. spicata was filtered through Whatman filter paper. The filtration process was repeated for several times and was placed for evaporation. Using rotary evaporator (Buchi Rota vapor R-200) at 45°C to evaporate the methanolic contents. The extract was placed in the laboratory under control conditions for further analysis.

Fractionation:

The dried material was dissolved in 80% aqueous methanol and portioned with hexane to remove the fatty materials. Remaining residue was dissolved in water and fractionation was carried out with chloroform, ethyl acetate in increasing order polarity. All the five extracts were dried using rotary evaporator. For further analysis all extracts were stored under control condition.

Antimicrobial Potential:

For antibacterial potential of fraction (3 mg/ml DMSO) of each was checked against bacterial species S. aureus (ATCC 29213), P. aeruginosa (ATCC 27853) and E.coli (ATCC 25922) by agar well diffusion method (14). The antibiotics Cefixime and Roxithromycin (1 mg/ml DMSO) were taken into consideration as a positive control. A 100 µl of each extract or antibiotics was poured into separate wells made in agar plates inoculated with cultured bacteria. After incubation of plates at 37°C for 24 hours period, clear zones of inhibition surrounding the wells were measured in mm.

Antifungal activity:

For antifungal activity 67µl of different extracts (12 mg/ml DMSO) were mixed with melted dextrose agar in test tubes. Test tubes were kept in a slating position and inoculated with fungi Aspergillus flavus (ATCC 9643), Aspergillus niger (ATCC 16404) and Aspergillus fumigatus (ATCC 204305) separately. Positive control had antifungal agent Terbinafine. After seven days of incubation at 28°C inhibition in linear growth of fungi in each test tube was determined.

$$\% \text{ inhibition growth} = (dc-dt/dc)\ x100$$

Negative control group was represented by c whereas sample growth is represented by t.

DPPH and Hyderogen Peroxide Free Radical Scavenging Activity:

The DPPH free radical scavenging assay was performed accordingly with that of Bibi et al., (2011) (15) whereas Hydrogen Peroxide Scavenging activity was done by the procedure given by Ruche, 1998 (16).

Cytotoxicity

In the initial step lymphocytes were isolated from blood samples in a saline phosphate buffer (pH 7.4) solution collected from healthy persons with an average age of 25 years old. The samples of blood were rotated in a centrifuge machine and after discarding supernatant the pellets were added with 3 ml of ficoll-hypaque solution. After rotating the samples again in a centrifuge machine (200 x g) lymphocytes were appeared above the ficoll-hypaque layer and were collected in a saline phosphate buffer solution. The isolated lymphocytes were then diluted with culturing medium (RPMI-1640, thermoscientific). A 10 µl of the culture medium was further added with trypan blue stain (0.2%) and taken to a haemocytometer equipped with a light microscope. After separating the stained (dead) and alive (non-stained) lymphocytes it was observed that more than 85% of the cells were in a living state. The culture media having lymphocytes was further added with more culture media (1×108 cells /ml) either pure or having 1, 10, 100 µg/ml of the extracts in separate containers and placed in an incubator at 37°C for complete

two hours. After centrifugation (200×g) for 15 minutes the pellet was collected and preserved in PBS (1×106) at -20°C. The pellet having lymphocytes was used for biochemical evaluation. The antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were extracted and determined. The method of Marklund ,1974 was followd for SOD determination by determining optical density of enzyme extract at 470 nm using a spectrophotometer (17). The SOD activity unit was demonstrated as mU/106 cells.

For catalase action dichromate/acetic acid reagent (1:3) was initially prepared by Sinha, et al (1972) (18). The H_2O_2 (0.2 M) was dissolved in 0.01 M phosphate buffer. Reaction mixture was comprised of 0.01 M phosphate buffer, tissue homogenate (100µl) and 2M H_2O_2 (400µl). After adding 2 ml dichromate/acetic acid reagent samples were incubated and analyzed for absorbance at 530 nm. The catalase activity was measured µM of H_2O_2 consumed/min/mg protein.

Using method of Carlberg and Mannervik,1975 peroxidase activity was measured. Homogenete (0.1 ml) was set containing guaiacol (100 µl), H_2O_2 (300 µl) and 50 mM phosphate buffer. Change in color after incubation of samples for 60 seconds optical density was measured at 470 nm. Unit of POD was as a change occurring in absorbance of samples as 0.01unit/minute.

Estimation of TBARS was made according to Li et al.,2010 by measuring absorbance of samples at 535 nm (19). The TBARS content unit was nano-moles per 106 cells.

The content of reactive oxygen species was estimated according to procedure of Hayashi et al., (2007) (20). A suspension of cells (5 µl) or pure H_2O_2 (taken as standard) was poured in a well plate containing sodium acetate buffer (pH 4.7). Mixtures of solutions were incubated at 37° C for 5 minutes and then added with DEPPD and FeSO4 (mixed in ratio of 1:12) solution (100 µl) and again incubated at 37° C for one minute. Samples absorbance was measured at 505 nm for a duration of three minutes at an interval f 3 minutes. The ROS concentration was reported as Units/106 cells.

Statistical analyses

Using two-way ANOVA data of cytotoxicity were analyzed. The data of anti-microbial activity were analyzed by ONE WAY ANOVA.

RESULTS:

Synthesis of AgNPs

To confirm the formation of AgNPs using plant extract, UV-Vis spectra was recorded in the range from 200nm to 800nm. The color of the silver solution upon addition of extract immediately changed to yellow, indicating the formation of AgNPs. Figure 2 shows the spectra recorded for the synthesized silver nanoparticles. The position of peak at position 450nm confirmed the synthesis of AgNPs. The color alteration noted by UV–vis spectroscopy, showed Ag capping ability with hydroxyl group present in plant extract. The result has been shown in Figure 1.

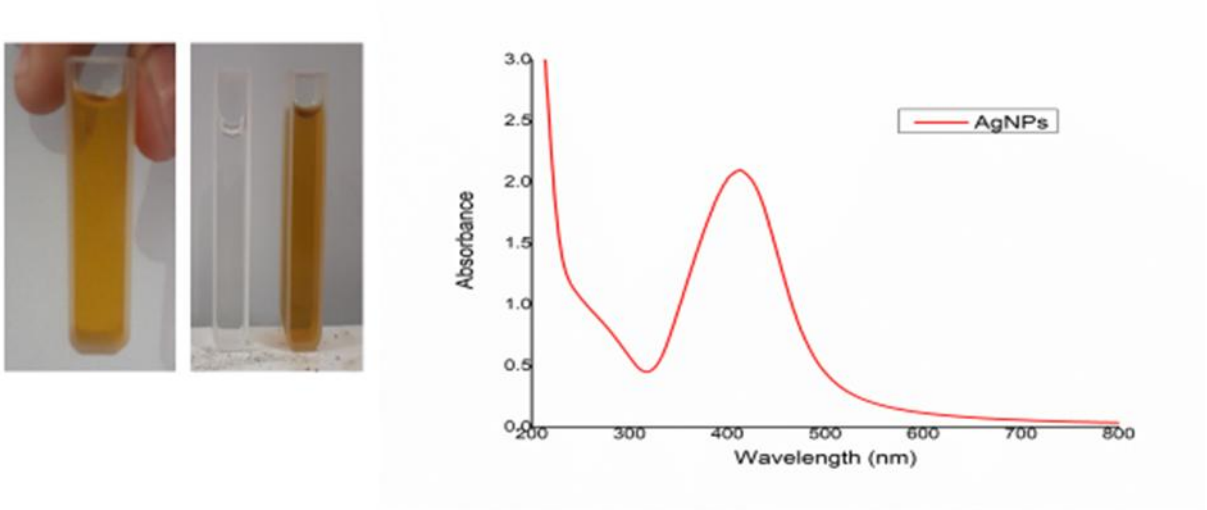

Figure 1. Calorimetric change of solution and UV-Visible spectrum of the synthesized AgNPS from extract of I. spicata plant.

Effect of pH on the synthesis of AgNPs

The formation of AgNPS was observed at a range i.e. pH 7, 8, 9, 10, 11 and 12. At pH 11 high absorbance was noted because the crude Ifloga spicata plant extract was stabilized and reduced to AgNPs therefore the pH 11 is selected for AgNPs from plant crude extract.

Extract amount effects on the synthesis of AgNPs

The plant extract amount concentration was evaluated in the range from 0.5 to 2ml. Figure 3 shows that with increase the quantity from 0.5 to 1ml, the intensity of absorption increases. When further the amount increase the intensity of absorbance then declines, showing the stability and reduction of Ag ions are almost completed at 1 ml extract concentration. Therefore, the 1ml amount is considered suitable concentration for AgNPs.

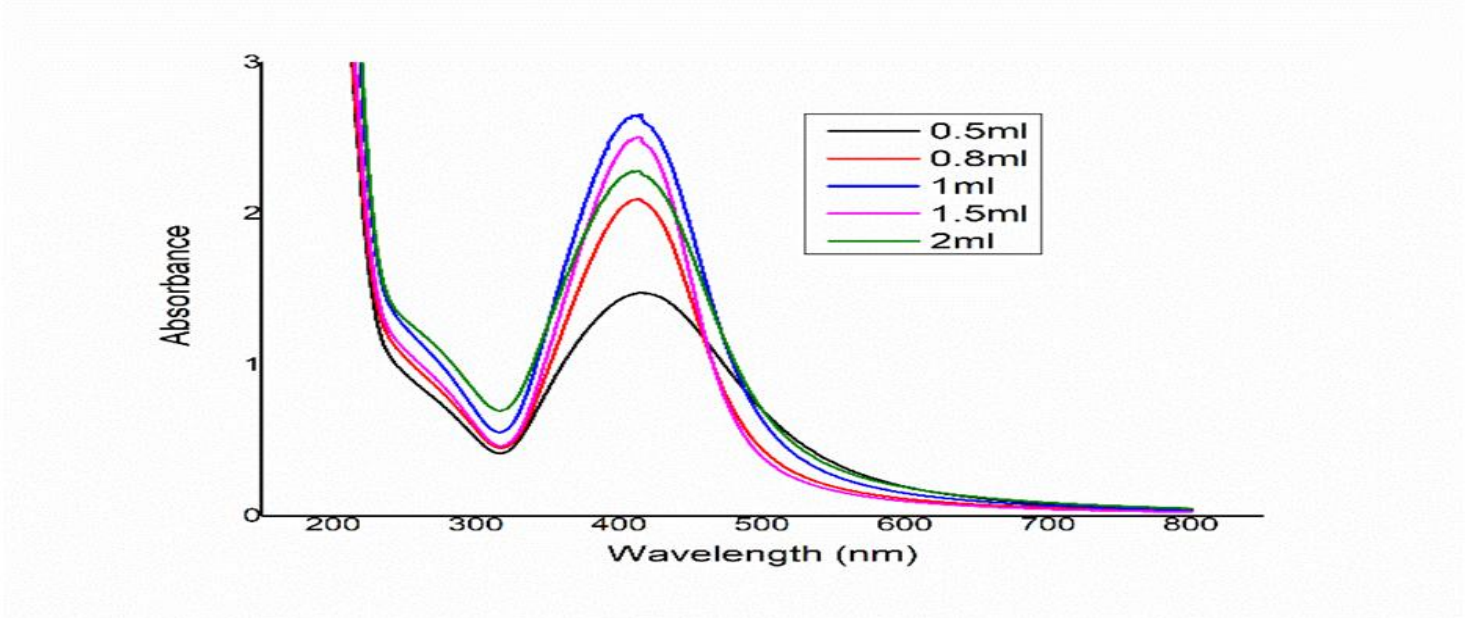

Figure 2. Effect extracts amount on the synthesis of AgNPs

FTIR of the synthesized AgNPs

FTIR analysis is important to investigate the presence of important biomolecules in the plant extract. The IR spectra at 3289.10 〖cm〗 ^(_1)show the stretching of –OH group in the plant extract with AgNps. Similarly, the peak found at 1603.13 〖cm〗 ^(_1), 1403.29 〖cm〗 ^(_1), 1259.19 〖cm〗 ^(_1), 1053.95 〖cm〗 ^(_1) showing stretching, c=c bond stretching and CH3 bending modes respectively.

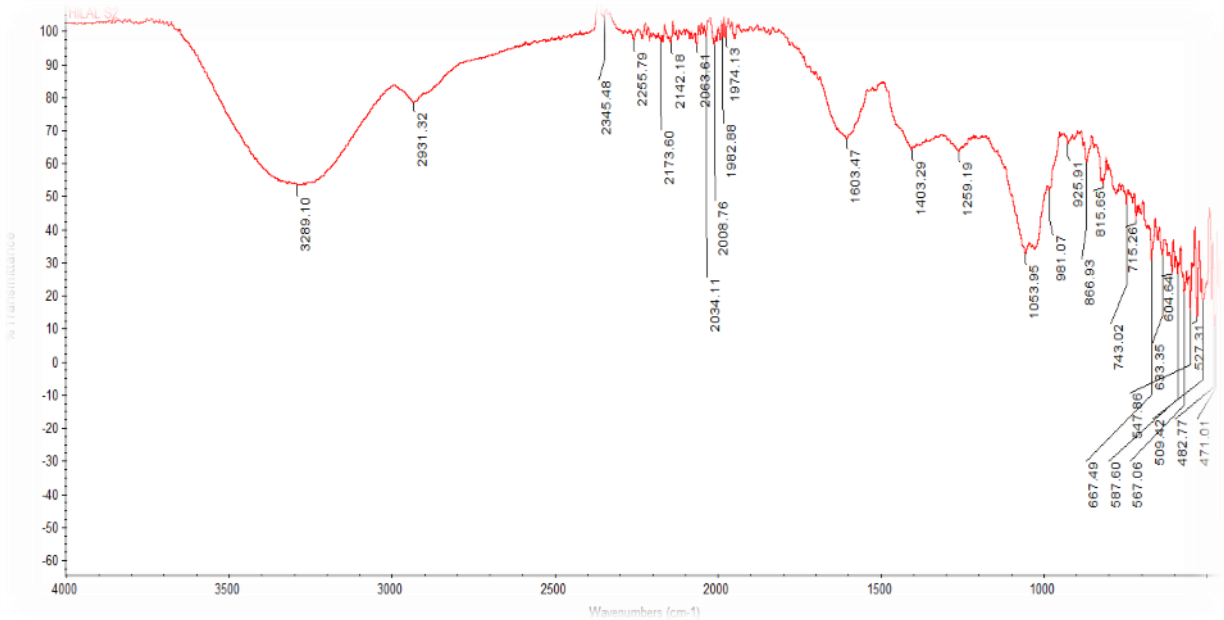

Figure 3. FTIR spectra for I. spicata plant extract before AgNPs synthesis.

Figure 4 shows the R spectra of AgNPs showing the decrease in wavelength due to stretching capability of important functional groups. The comparative data showed that AgNPs are synthesized.

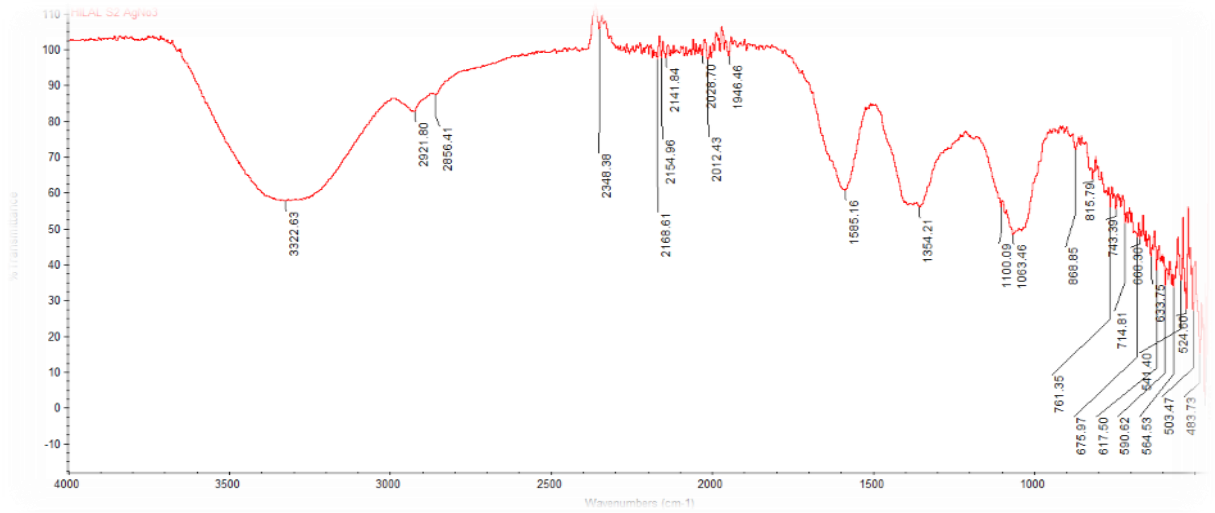

Figure 4. FTIR spectra of AgNPs synthesized from I. spicata extract.

Scan Electron Microscope (SEM)Analysis

The SEM study was carried out to determine physical nature and morphology of synthesized AgNPs. The scanned electron micrograph of synthesized silver nanoparticles are presented in Figure 5, showing 1µm size of the AgNPs.

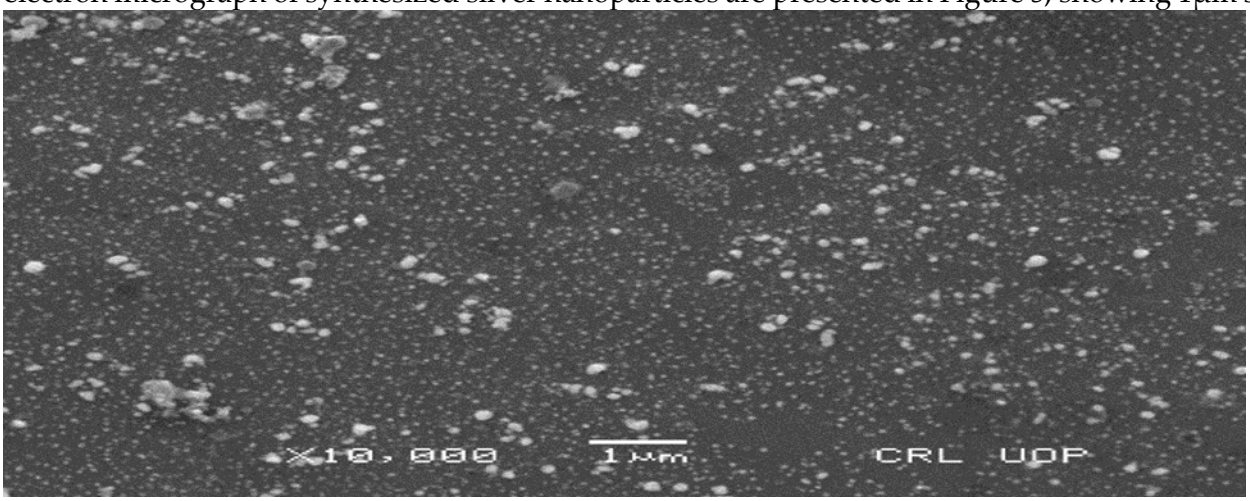

Figure 5. SEM analysis of AgNPs

X-Ray Diffraction (XRD)

X-ray diffraction study was carried out to confirm the crystalline nature of the AgNPs. The XRD blueprint, (Figure 6), reveal numbers of Bragg reflections at 2θ values of 38.21(111), 346.29(200), 64.64(220) and 77.55(311) sets of lattice plane. These planes demonstration based on the face-centered cubic structure of silver. The XRD pattern thus showing the crystalline structure of the AgNPs.

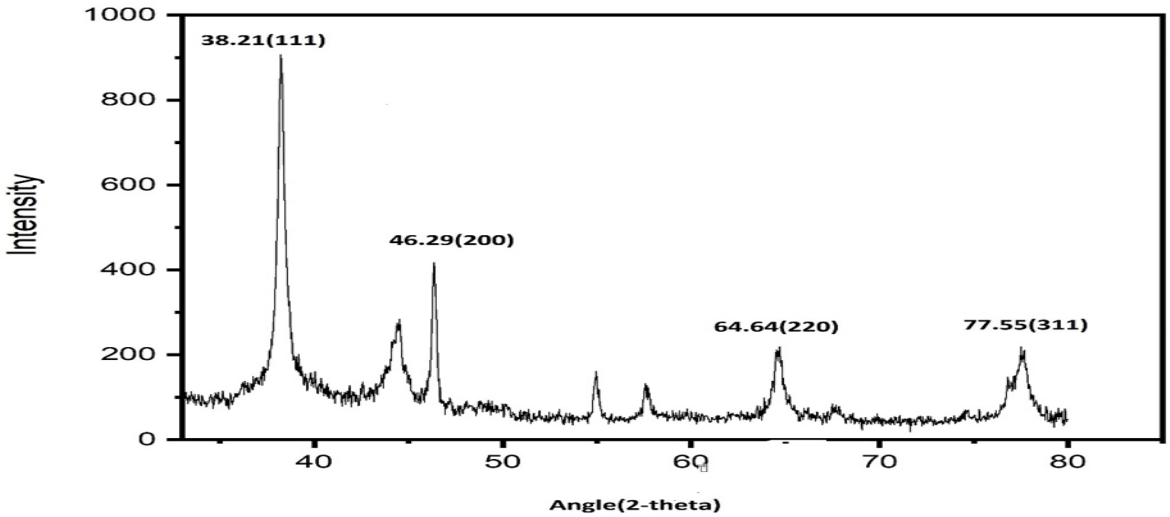

Figure 6. X-Ray Diffraction (XRD) analysis of AgNPs prepared of I. spicata.

DPPH Scavenging Activity

The synthesized AgNPs showed potent antioxidant property as compared to plant extract. In this assay various concentrations of crude extract AgNPs were used. The maximum DPPH scavenging potential was shown by AgNPs at 50µg/ml as compare to crude plant extract. Figure 7 showing concentration effect of both AgNPs and crude extract.

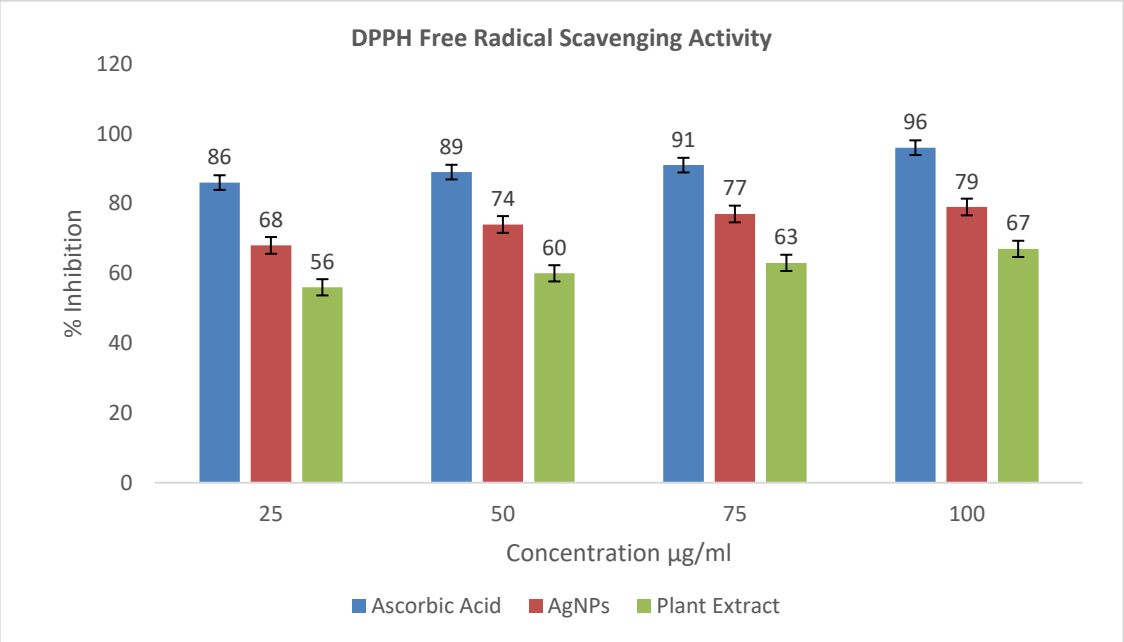

Figure 7. DPPH free radical scavenging activity of synthesized AgNPs and I. spicata extract.

Hydrogen peroxide scavenging activity

Plant extract and AgNPs have free radicals scavenging property. In this assay four different concentrations of AgNPs and crude extracts were used. The increase in concentration increase the scavenging ability of both AgNPs and crude extract. However, the AgNPs showing more scavenging effect as compared to crude plant extract of the same plant I. spicata. Figure 8 showing that at concentration 80 both plant and AgNPs maximum scavenging activity.

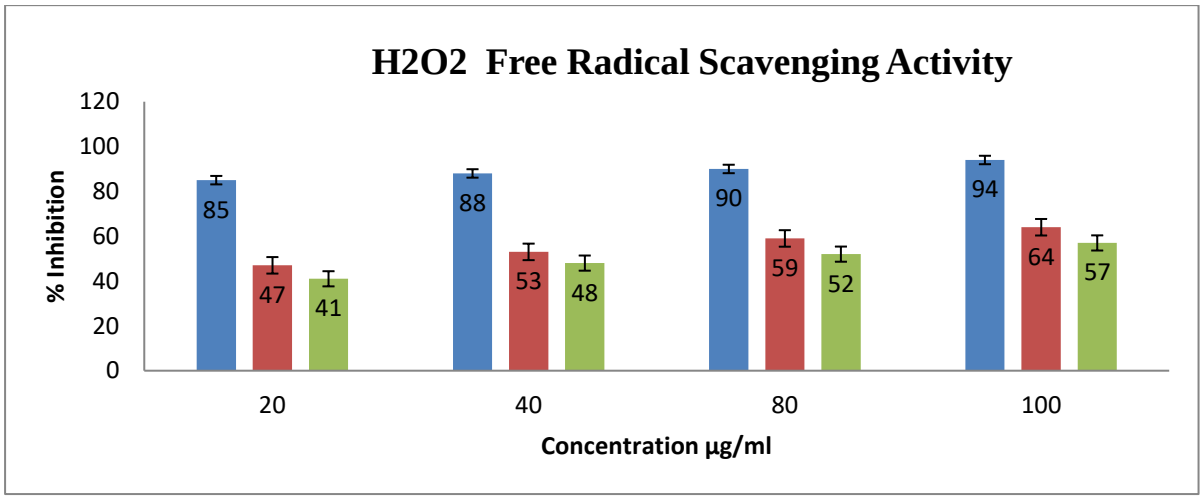

Figure 8. H_2O_2 free radical scavenging of AgNPs and I. spicata plant extract.

ABTS Free Radical Scavenging Activity

In this assay ABTS free radical scavenging potential was shown by both plant based silver nanoparticles and crude extract. In comparative study the AgNPs exhibit maximum scavenging potential as crude extract of the same plant. Different concentrations were cheeked including 25µg/ml, 50µg/ml,75µg/ml and 100µg/ml. The most potent inhibitory effect was shown by AgNPs at 50 and 75µg/ml respectively.

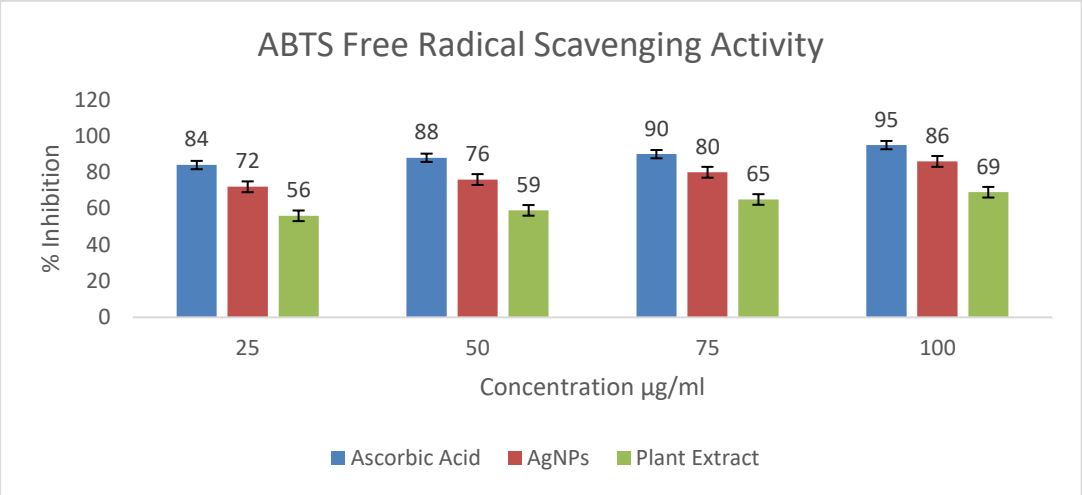

Figure 9. ABTS free radical scavenging activity of AgNPs and I. spicata plant extract.

Antibacterial Activity (mm)

Table 1 shows antibacterial activity of plant extract of I. spiccata and synthesized AgNPs against four different bacterial strains namely Klebsiella pneumoniae, Escherichia coli , Acetobactor orientalis and Staphylococus aureus. Different concentrations of both AgNPs and crude extract were used. However, it is reported that both AgNPs and crude extract exhibit maximum inhibitory potential at 20 µg/ml against A. orientalis, S. aureus and E. coli. The zone of inhibition is measured in (mm).

Table: 1 Antibacterial activities of plant extract and synthesized AgNPs of I. spicata.

Concentrations	*K. pneumonia*	*E. coli*	*A. orientalis*	*S. aureus*
Erythromycine 100 µg/mL	47±0.78	39.5±0.89	44.3±0.35	44±0.76
I .spicata 20µg/mL	21.5±0.70	27.5±0.23	17.6±0.55	20.8±0.76
AgNPs 20 µg/mL	13.5±0.70	19.8±0.43	24.6±0.34	26.6±0.14
I. spicata 40 µg/mL	6.64±0.95	13.7±1.27	15±0.49	19.8±0.65
AgNPs 40 µg/mL	14.7±0.73	16±9.56	18±0.76	22.1±0.23
I .spicata 80 µg/mL	4.2±0.34	10.5±1.22	16.6±0.84	17.5±0.50
AgNPs 80 µg/mL	12.5±0.37	14±1.16	12.3±0.97	21.6±0.45
I .spicata 100 µg/mL	7.8±1.26	11.8±0.67	19.3±0.96	21.6±0.22
AgNPs100 µg/mL	28±0.46	21.8±1.46	17.5±0.06	12.8±0.84

Cytotoxicity screening of Ifloga spicata synthesized AgNPs

The brine shrimp assay was performed for cytotoxic analysis of AgNPs. The data were collected after 24 hours. The results of the current study show that I. spicata AgNPs posses' significant cytotoxicity potential at a concentration 10µg (Figure 10).

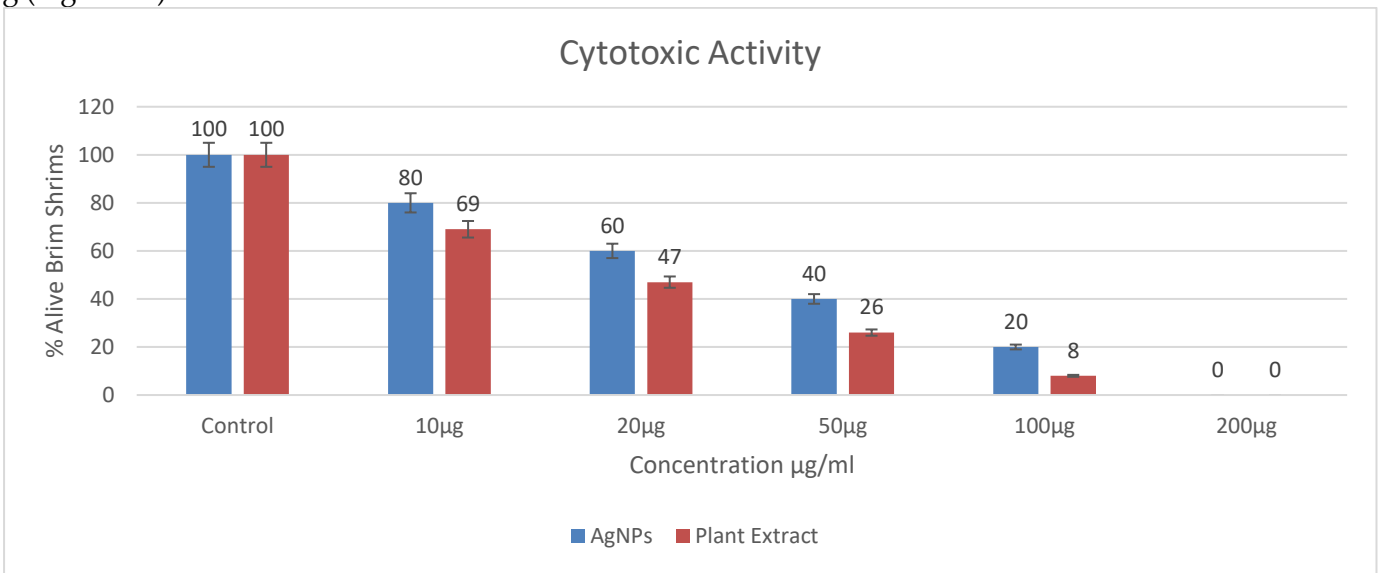

Figure 10. Cytotoxic effect of synthesized AgNPs

Effect of Plant based AgNPs on ROS content of lymphocytes

Under normal conditions extract of plant based AgNPs has no adverse effect on ROS contents in blood lymphocytes. The ROS level in blood lymphocyte was increased due to H_2O_2 stress. The elevated ROS level due to H_2O_2 treatment was considerably recovered by different fractions of I.spicata based AgNPs. Most effective treatments were found at 5ug/ml and 50 ug/ml different concentrations. The fractions ethyl acetate and n-hexane was significantly recovered ROS level ($p>0.05$).

Effect of Ifloga spicata on Antioxidant Enzymes Activity of Blood Lymphocytes

Due to H_2O_2 stress the blood lymphocytes SOD, CAT and POD activities were significantly deceased as compared to normal control ($p<0.05$). Under normal conditions the plant based AgNPs having no side effect on the SOD, CAT and POD activities of blood lymphocytes. However, the various fractions of plant based AgNPs at 5 µg/ml and 50 µg/ml significantly increased antioxidant enzymes activity (SOD, CAT and POD) potential. Despite the other fractions, methanol and aqueous fractions were significantly more effective in recovering the above enzymes activities as shown in Tables 2-4.

Effect of Plant based AgNPs on TBARS of blood lymphocytes

The blood lymphocytes were observed with higher TBARS activity (nano-moles per 106 cells) when treated with H_2O_2 as compared to control group. However, the different fractions of plant based AgNPs recovered the TBARS contents. 5 ug/ml and 50 ug/ml were the most effective concentration at which blood lymphocytes recovered maximum TBARS contents. As shown in Table 5 the two AgNPs fractions, ethyl acetate and chloroform were more significantly recovered the induced TBARS oxidative stress ($p<0.05$).

Table 1. Showing the effect of plant based AgNPs on ROS content (Units / 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress.

Treatment	Fractions					Mean
	E1(Methanol	E(Ethylacetate)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	0 .2220 ±0.03g-h	0 .2560±0.04g	0 .2560±0.04g	0 .2560 ±0.03g	0 .2560 ±0.03g	0 .256±0.02de
100µM Hydrogen per oxide	0 .3950 ±0.6a	0 .3950 ±0.03a	0.3950 ±0.017a	0 .3950 ±0.06a	0 .3950±0.02a	0 .3950±0.017a
AgNPs 0.5 µg/ml	0 .2433±0.4 g	0 .486±0.06e	0 .2643±0.05f	0 .2399±0.01g	0 .2650±0.10f	0 .2582±0.032d
AgNPs 5 µg/ml	0 .2401±0.16 h	0 .2529 ±0.04e	0 .2337 ±0.04h-j	0 .2379±0.3g	0 .2360±0.02g	0 .2393±0.06de
AgNPs 50 µg/ml	0 .2111±0.2 i	0 .2423±0.02g	0 .2357 ±0.03g-j	0 .2189±0.05i	0 .2327±0.05h	0 .2281±0.04 e
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	0 .3753 ± 0.014ab	0 .3889±0.024ab	0 .3679±0.07 ab	0 .3639±0.02ab	0 .3559±0.04a	0 .3694 ±0.02b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	0 .2837±.0.2 e	0 .36457±0.03ab	0 .3475±0.02a-d	0 .3124±0.022d	0 .3449 ±0.02b	0 .3298±0.012c
100µM Hydrogen per oxide + AgNPs 50 µg/ml	0 .2733 ±0.21e	0 .3795±0.03ab	0 .3621 ±0.03a-c	0 .3148 ±0.4c	0 .3495 ±0.5ab	0 .3351 ±0.14c
Mean	0 .2835±0.02 c	0 .3185 ±0.03a	0 .2964 ±0.05ab	0 .2899 ±0.08b	0 .2932±0.04ab	

± showing standard error value.

Table 2. Showing the effect of Plant based AgNPs on CAT activity (milli Units / 10^6 cells) of human blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	3.9211±0.14b-e	3.9211± 0.16b-e	3.8100±0.13b-e	3.9211±0.14b-e	3.91211 ±0.14b-e	3.9211 ±0.12a
100µM Hydrogen per oxide	1.8571±0.12n	1.8571±0.13n	1.8571±0.112n	1.8571±0.15n	1.8571 ±0.12n	1.8571 ±0.114c
AgNPs 0.5 µg/ml	4.4671±0.305ab	3.86110±0.16b-e	3.4671 ±0.14e-h	3.7811±0.17b-e	3.8183±0.14b-e	3.8829 ±0.16a
AgNPs 5 µg/ml	4.3411±0.12abc	3.6611±0.25d-f	3.7291±0.16c-e	3.969±0.13b-e	3.9870 ±0.05b-e	3.9430 ±0.16a
AgNPs 50 µg/ml	4.7991±0.13a	3.6450±0.12d-e	4.2371±0.15a-d	3.8269 ±0.4b-e	3.9531±0.14b-e	4.0143 ±0.14a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	2.9131 ±0.16 f-j	1.8990 ±0.0121n	1.9420 ±0.14n	2.5581±0.12j-m	2.7821±0.13i-m	2.499 ±0.13b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	3.5711 ±0.14d-g	2.1199±0.10mn	2.15620±0.15nm	2.3911±0.15j-m	2.82540 ±0.17-l	2.6070 ±0.14b
100µM Hydrogen per oxide + AgNPs 50 µg/ml	3.4270 ±0.11e-i	2.08940 ±0.011mn	218980 ±0.11k-n	2.3920±0.02j-m	2.8523 ±0.03j-k	2.6455 ±0.12b
Mean	3.6998 ±0.13a	2.8449 ±0.17c	2.9885±0.12c	3.0197 ±0.12bc	3.2899 ±0.011b	

± showing standard error value.

Table 3. Showing the effect of Plant based AgNPs on POD activity (nmol / 10^6cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethyl acetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	5.9930 ±0.123ab	5.9931±0.32ab	5.9931± 0.14ab	5.9931±0.34ab	5.9931 ±0.04ab	5.9931 ±0.43b
100µM Hydrogen per oxide	3.9391 ±0.14de	3.9391±0.12de	3.9391 ±0.23de	3.9291±0.22de	3.9391 ±0.32de	3.9391 ±0.13cd
AgNPs 0.5 µg/ml	6.1371 ±0.134ab	4.7571±0.4cd	6.999± 0.11a	6.9999±0.15a	6.3285 ±0.15ab	6.2445 ±0.16ab
AgNPs 5 µg/ml	5.9511 ±0.144ab	6.288 ±0.31ab	6.0645± 0.10ab	6.2711 ±0.32ab	6.645 ±0.34a	6.2943±0.51ab
AgNPs 50 µg/ml	7.1493 ±0.12a	6.6911 ±0.44a	6.4485±0.12a	6.3911±0.22ab	6.8150 ±0.16a	6.724±0.125a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	3. 3841 ±0.13de	3.4995±0.22e	3.49999± 0.15e	3.481±0.23e	3.3291 ±0.23e	3.4329±0.12d
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	3.7351±0.16de	4.3373 ±0.14c-e	3.6791± 0.14de	3.9389 ±0.13c-e	4.3751 ±0.35c-e	3.9555 ±0.22cd
100µM Hydrogen per oxide + AgNPs 50 µg/ml	4.3631 ±0.23c-e	3.4460 ±0.02de	3.7890± 0.15de	3.5937±0.12de	5.1871±0.432bc	3.9882 ±0.13c
Mean	5.1526 ±0.06ab	4.8862±0.001b	4.9678 ±0.13ab	4.9938±0.34ab	5.3569 ±0.33a	

± showing standard error value.

Table 4. Showing the effect of Plant based AgNPs on SOD activity (milli Units / 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	10.355 ±0.34d	10.355±0.33de	10.355 ±0.12d	10.355 ±0.32d	10.355 ±0.47d	10.355 ±0.23b
100µM Hydrogen per oxide	5.147 ±0.43k	5.147 ±043k	5.047±0.14k	5.147 ±0.25k	5.047±022k	5.147±144 e
AgNPs 0.5 µg/ml	11.759±0.43a-d	11.235± 0.11a-e	10.263±0.17e	10.221 ±0.34ef	10.720 ±0.32b-e	10.838 ±0.13ab
AgNPs 5 µg/ml	11.395±0.44a-e	10.663±0.5cde	11.267 ±0.66a-e	10.981±0.14b-e	12.193±0.01a b	11.299 ±0.35a
AgNPs 50 µg/ml	11.859±0.54ab	11.588 ±0.47a-e	10.769±0.7b-e	8.6779 ±0.44g	12.585 ±0.10a	11.018±067a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	7.409±0.55ijk	7.027±0.33ijk	5.503±0.34jk	5.907±0.7i-k	7.403 ±0.77g-i	6.312±0.53d
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	10.688 ±0.55g	9.327 ±0.34h	8.663±0.55i-j	7.495±0.63k	6.881±0.44ij	7.969±0.39c
100µM Hydrogen per oxide + AgNPs 50 µg/ml	8.6587 ±0.44gh	6.373 ±0.32ij	6.223 ±0.01i-j	5.977±0.33i-k	8.746±0.66fg	7.1216 ±0.2c
Mean	9.2577±0.12a	8.4868±0.33b	8.258 ±0.33bc	7.8563±0.26c	9.2564±0.33a	

± showing standard error value.

Table 5. Showing the effect of Plant based AuNPs on TBARS (nano-moles/ 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	0 .7971±0.012f	0 .7971 ±0.013f	0 .7971 ±0.22f	0 .7971 ±0.11-j	0 .7971 ±0.13f	0 .7971 ±0.2a
100µM Hydrogen per oxide	1 .0255±0.11a	1 .0255±0.10a	1 . 0255±0.11a	1 . 0255±0.12aA	1 . 0255±0.014a a	1 . 0255 ±0.11a
AgNPs 0.5 µg/ml	0 .6463±0.03k-l	0 .7421±0.22h-j	0 .7633 ±0.23h-j	0 .73470.23h-l	0 .7629 ±0.0031h	0 .7299 ±0.011e
AgNPs 5 µg/ml	0 .5951±0.003lm	0 .6199 ±0.41i-	0 .7945±0.11g	0 .7617 ±0.08h	0 .6899±0.11i	0 .7142 ±0.04e
AgNPs 50 µg/ml	0 .5763 ±0.01m	0 .5985 I±0.02i-m	0 .6790 ±0.30j-m	0 .7520±0.20h-k	0. 6690 ±0.001j	0 .6749 ±0.04e
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	0 .9145±0.04abc	0 .9185±0.57abc	0 .9849±0.01ab	0.8853±0.004bcd	0 .7575±0.02d-h	0 .8981 ±0.12b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	0 .8417±0.012c-f	0 .9213 ±0.012abc	0 .8943±0.62abcd	0 .8593±0.016cd	0 .7651 ±0.013d-h	0 .8565 ±0.16bc
100µM Hydrogen per oxide + AgNPs 50 µg/ml	0 .8360 ±0.017e	0 .9544 ±0.017cd	0 .9230 ±0.11c	0 .9700±0.013ab	0 .8278±0.33e	0 .9202 ±0.23c
Mean	0 .8168±0.14b	0 .8849 ±0.015a	0 .8980±0.11a	0 .894 ±0.11a	0 .881±0.11b	

± showing standard error value

DISCUSSION

The development of easy, reliable and eco-friendly methods helps to increase interest in the synthesis and application of nanoparticles that are beneficial for mankind. Speedy biosynthesis of stable gold, silver and bi-

metallic Ag/Au core shell nanoparticles with 20 g of Azadirachta indica leaf biomass and 1mM aqueous $AgNO_3$, with a 90% reduction of the metal ions within 4 hrs. The dissimilarity in the rates of bio-reduction observed may be due to the differences in the activities of the enzymes present in the A. indica and I. herbstii aqueous leaf extracts. S. Ankanna et al.,2010 also reported the reduction of silver ion into silver particles when mixed to plant extracts could be followed by a color change (21). The silver nanoparticles exhibited a dark yellowish-brown color in the aqueous solution due to the surface plasmon resonance phenomenon.

In current study plant based AgNPs have been synthesized. The FT-IR study showed that the synthesized AgNPs from Ifloga spicata plant extract contains various functional groups. AgNPs were characterized by using UV-Visible spectroscopy, (FTIR), (EDS), and (TEM). The nanoparticles size were spherical determined by SEM study. Antibacterial activities of the synthesized AgNPs were also investigated against Escherichia coli. The antibacterial properties and silver release profiles were evaluated after interacting with phosphate-buffered saline or with serum in vitro. The study showed promising results directing towards therapeutic potential of the studied plant extracts, however further studies will be required to confirm targeted used and drug development.

CONCLUSION

Nanotechnology provides innovative approach to test and develop new synthesized drugs formulation based on biosynthesis nanoparticles with different biological potentials such antioxidant and antimicrobial potentials. The physical parameters such as size and shape are important to enhance the antimicrobial potentials. The plant-based silver nanoparticles are eco-friendly with low cost and more potent antioxidants and anticancer potentials.

Acknowledgment:

The author is thankful to University of Science and Technology Bannu K.P Pakistan to provide research opportunity.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by the local Ethical Review Committee.

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Original Article

THE ASSOCIATION BETWEEN DIETARY HABITS, SLEEP PATTERNS, PHYSICAL ACTIVITY, AND OBESITY AMONG MEDICAL STUDENTS

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DOI: 10.38106/LMRJ.2025.7.2-05
Received: 17.05.2025
Accepted: 20.06.2025
Published: 30.06.2025

ABSTRACT:
Obesity represents a significant global health concern, impacting nations across all economic strata. Medical students face heightened risks of adopting detrimental habits due to the pressures of academic life, which can result in weight gain and associated health problems. The purpose of this study was to evaluate the prevalence of obesity among medical students at Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan and investigate its association with lifestyle factors including eating habits, physical activity levels, and sleep patterns. This was cross-sectional study including 148 medical students. Data was collected through a structured questionnaire assessing participants' dietary habits, physical activity, and sleep patterns. The mean Body Mass Index (BMI) was 23.53 kg/m²; 20.3% were overweight and 6.8% obese, more common in males. Fast-food intake was higher in these groups. No strong link existed between BMI and sleep, though obese students reported to have irregular sleep patterns. Physical activity was low, with 78.35% of overweight/obese students exercising 1–2 times weekly or not at all. This study identified a significant association between BMI and lifestyle factors among medical students, with males showing a higher tendency toward overweight and obesity. Elevated BMI was linked to frequent fast-food consumption, low levels of physical activity, and irregular sleep patterns. These findings underscore the need for targeted interventions that promote healthy eating habits, regular physical activity, and improved sleep hygiene.

Keywords: Obesity, BMI, lifestyle factors, physical activity, dietary habits, sleep patterns

INTRODUCTION

The incidence of obesity is a growing concern worldwide, affecting both developed and developing countries. Historically viewed as a condition primarily affecting affluent nations, but it is increasingly becoming prevalent in low- and middle-income countries undergoing urbanization and subsequent lifestyle changes. In the year 2005, projections indicated that approximately 937 million adults worldwide suffered from excess weight, with 396 million of these individuals meeting the criteria to fall in the category of obese adults. At that time, approximately 23% of the global population was overweight, and 9.8% were considered obese. If trends continued, it was estimated that by 2030, there would be 2.16 billion overweight (38% of the world's population) and 1.12 billion obese (20%)(3).

In 2022, the World Health Organization (WHO) released statistics indicating that approximately 2.5 billion adults globally, constituting 43% of the adult population, were categorized as overweight. Furthermore, projections for 2023 indicated that approximately 890 million adults, or 16% of the worldwide adult population, were experiencing obesity(4). Obesity is increasingly acknowledged as a complex issue influenced by genetic, environmental, and behavioral elements. This encompasses the intake of energy-dense, nutrient-poor foods, sedentary life style, and impulsive expenditure of energy. Furthermore, obesity significantly contributes to the risk profile for non-communicable diseases (NCDs), including cardiovascular conditions, diabetes, chronic respiratory diseases, and certain cancers (5, 6).

The public health impact of obesity is gaining recognition in Pakistan. The country has seen a change in eating and exercise habits resulting from urbanization, and the rate of obesity has dramatically increased. Lifestyle factors such as irregular eating habits, physical inactivity, and poor sleep patterns contribute to weight gain among young adults, especially medical students(7). Medical students are particularly susceptible to unhealthy behaviors that can lead them to obesity-related comorbidities later in life due to the psychosocial academic pressures and stress(8). Considering these issues, this study was designed aiming to assess the prevalence of obesity among medical students and explore how factors such as eating habits, physical activity, and sleep patterns correlate with obesity indicators like Body Mass Index (BMI). These correlation patterns need to be determined to create effective intervention programs to promote healthier lifestyles among the population of medical students, ultimately

minimizing the long-term burden of obesity-associated health conditions in Pakistan. In addition, the study aimed to bridge the existing gap in the existing body of knowledge and enhance awareness programs for the population of medical students. Healthier lifestyles among future doctors could have long-term public health consequences.

METHODS:

The research was conducted at Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, Pakistan, from July 2024 to December 2024. This was a cross-sectional study including medical students studying in any year from first to final, using a structured questionnaire. The questionnaire included information regarding their exercise habits, sleep patterns, and dietary behaviors. Initially, 200 students were considered for inclusion; however, after applying the inclusion and exclusion criteria, the final participant pool was narrowed to 148 individuals, selected through a convenience sampling method.

Statistical analyses

Data were processed by using the Statistical Package for Social Sciences version 22.0 for Windows, presenting the results as means ± standard deviations (SD) and percentages. The analysis of variance for continuous variables between groups was conducted using independent t-tests, whereas the application of chi-square tests evaluated categorical data. Furthermore, correlation analysis explored the associations among physical activity, sleep duration, nutritional behaviors, and Body Mass Index (BMI). A p-value of less than 0.05 was designated as statistically significant.

RESULTS:

A total of 148 medical students were recruited in our study, with a median age of 20.34 years. The median height of the participants was 165.61 cm, while the median weight of the participants was 63.03 kg, resulting in a mean BMI of 23.53 kg/m². Out of 148 students, 16 students (10.8%) BMI falling in the category of underweight, 92 students (62.2%) had normal weight BMI, 30 students (20.3%) had overweight BMI, and 10 students (6.8) were obese (Table 1). Overweight and Obese students were predominantly male, and there was significant association was indicated by chi-square test between BMI and gender, only male students were obese, while no any female student found obese, 86.7% male students were overweight BMI and 13.3% female students were overweight BMI, and 66.3% male students were normal weight BMI and 33.7% female students were normal weight BMI, whereas 68.8% male students were underweight BMI and 31.2% female students were underweight.

A total of 56.65% of students classified as overweight or obese reported experiencing recent fluctuations in their weight, either as loss or gain. However, 43.35% of students did not report any recent changes in their weight. 78.35% obese and overweight BMI students were engaging in exercise daily 1 to 2 days per week or no exercise while 21.65% obese and overweight BMI students were engaging with 4 to 6 days per week, and 77.2% normal weight BMI students were engaging 1 to 2 days per exercise or no exercise, while 13.1% normal weight BMI students, were engaging themselves in 4 to 6 days per week exercise (Table 2), and there was significant relation between BMI and weight (p-value= 0.03), overall the obese or overweight are not reported to have daily exercise, while normal weight BMI students were more likely to engage in daily or frequent physical activity. There was no significant association found between BMI and sleep patterns (p-value =0.127). However, it was observed that students with a normal or overweight BMI typically achieved 7 to 8 hours of sleep each night. In contrast, those classified as obese exhibited more erratic sleep patterns, frequently logging either less than 6 hours or more than 8 hours of sleep.

Among students, 40% of those classified as obese and 76.7% of those who are overweight with respect to their BMI indulge in fast food at least once or twice a week. Obese students, however, show a tendency towards a more frequent pattern, consuming fast food daily or three to four times a week. In contrast, 56.5% of students with a normal BMI also partake in fast food once or twice weekly, yet 22.8% abstain entirely from fast food consumption each week and 20.7% of students with a normal BMI report eating fast food three to five times per week (Table 3).

Table 1. Distribution of Body Mass Index (BMI) categories

BMI Category	Male	Female	Total
Obese (>30 BMI)	10 (100.0%)	0 (0.0%)	10 (6.8%)
Overweight (25-29.9 BMI)	26 (86.7%)	4 (13.3%)	30 (20.3%)
Normal Weight (18.5-24.9 BMI)	61 (66.3%)	31 (33.7%)	92 (62.2%)
Underweight (<18.5 BMI)	11 (68.8%)	5 (31.2%)	16 (10.8%)
Total	108 (73.0%)	40 (27.0%)	148 (100.0%)

Table 2. Relationship between BMI categories and frequency of physical activity among students

BMI Category	None	1-2 Days	3-4 Days	5-6 Days	Every Day	Total
Obese (>30 BMI)	3 (30.0%)	5 (50.0%)	1 (10.0%)	1 (10.0%)	0 (0.0%)	10 (6.8%)
Overweight (25-29.9 BMI)	8 (26.7%)	15 (50.0%)	7 (23.3%)	0 (0.0%)	0 (0.0%)	30 (20.3%)
Normal Weight (18.5-24.9 BMI)	39 (42.4%)	32 (34.8%)	9 (9.8%)	3 (3.3%)	9 (9.8%)	92 (62.2%)
Underweight (<18.5 BMI)	5 (31.3%)	6 (37.5%)	2 (12.5%)	3 (18.8%)	0 (0.0%)	16 (10.8%)

Table 3. Fast Food Consumption Patterns Across Different BMI Categories

BMI Category	Never	1-2 Times	3-4 Times	5-6 Times	Daily	Total
Obese (>30 BMI)	2 (20.0%)	4 (40.0%)	3 (30.0%)	0 (0.0%)	1 (10.0%)	10 (6.8%)
Overweight (25-29.9 BMI)	0 (0.0%)	23 (76.7%)	3 (10.0%)	1 (3.3%)	3 (10.0%)	30 (20.3%)
Normal Weight (18.5-24.9 BMI)	21 (22.8%)	52 (56.5%)	15 (16.3%)	2 (2.2%)	2 (2.2%)	92 (62.2%)
Underweight (<18.5 BMI)	3 (18.8%)	12 (75.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	16 (10.8%)

DISCUSSION

Our study identifies significant correlations between BMI and lifestyle factors such as gender, exercise habits, and dietary choices. The higher prevalence of obesity and overweight among male students is consistent with previous research, which indicates gender differences in body composition and metabolism (9). Further studies have shown that male medical students tend to have higher rates of obesity and overweight compared to their female counterparts (10). Research conducted in Greece found that a substantial proportion of both male and female medical students—specifically, 40% of males and 23% of females—had a BMI of 25.0 kg/m² or higher (11).

Most students classified as overweight or obese reported experiencing changes in their weight, likely due to efforts to manage their weight or due to inconsistent physical activity and dietary habits. However, our findings did not show a significant link between sleep duration and BMI. While other studies suggest that irregular sleep patterns contribute to obesity (12), our research found that although obese students exhibited more irregular sleep patterns, this factor was not significantly associated with BMI status.

Physical activity levels also varied significantly across the different BMI groups. Overweight and obese students engaged in less physical exercise compared to their normal-weight peers. This finding aligns with existing literature that emphasizes the importance of exercise in maintaining a healthy BMI and preventing weight gain (13). Moreover, low levels of physical activity have been identified as a primary contributor to obesity, with studies indicating that reduced energy expenditure plays a more critical role than increased food intake in weight gain development (14).

Eating habits were found to significantly influence BMI, especially regarding fast food consumption. Obese individuals showed a higher tendency to consume fast food compared to those with a normal weight. The link between fast food consumption and obesity is well established, mainly due to excessive caloric intake, poor nutritional content, and the convenience of fast food, all of which contribute to weight gain (15). A longitudinal study on eating patterns revealed that individuals who consumed fast food more than twice a week gained more weight over time and had higher levels of insulin resistance (16). Additionally, prolonged sedentary behaviors, such as excessive screen time, were linked to a greater likelihood of obesity. The Nurses' Health Study found that an additional two hours of television watching was associated with a 23% increase in the likelihood of obesity and a 14% rise in the risk of developing diabetes (17). In our study, students reported a median screen time of 2 hours daily, which may contribute to sedentary behavior and weight gain. These findings highlight the need for health promotion initiatives, especially those that encourage a balanced diet, regular physical activity, and quality sleep to support weight management and overall well-being among medical students.

CONCLUSION

There was a significant association between lifestyle factors and the BMI of medical students, with a higher incidence of obesity and overweight observed in male students. Frequent fast food consumption, irregular sleep patterns, and excessive screen time were found to contribute to higher BMI levels. Additionally, physical activity levels were notably low, especially among obese and overweight students, underscoring the urgent need for targeted interventions to promote healthier lifestyle choices.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by the local Ethical Review Committee.

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Liaquat Medical Research Journal (LMRJ)
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Original Article

EVALUATION OF FAMILY STRUCTURE AND PARENTAL LOSS AND PEER BULLYING AS RISK FACTORS FOR CHILDHOOD DEPRESSION: A SCHOOL-BASED DESCRIPTIVE-CROSS-SECTIONAL STUDY

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DOI: 10.38106/LMRJ.2025.7.2-06
Received: 12.04.2025
Accepted: 15.06.2025
Published: 30.06.2025

ABSTRACT:

Depressive symptoms after parental loss are a natural phenomenon, tagged with loss of support, financial constraints and disruptive routine. The loss of parent may be further enhanced in cases of painful divorces. At the same time bullying is considered as a major psychological trauma for growing children. This study was conducted to evaluate depression in school children following parental loss resulting in change in family structure and also with the history of peer bullying. This descriptive cross-sectional study was conducted in school going children. A total of 1000 children were evaluated aged between 5 to 15 years. Out of these 42 children had a history of parental loss within last two years while 12 had lost their parents more than two years ago. Depression was reported in 61 students and showed significant association with loss of parent, both by death or divorce. Bullying also showed significant association with depression. In conclusion, children having history of parent loss and bullying showed significant association of depression. Therefore, mental health support for these both group of children should be considered even if they do not show apparent signs of depression.

Keywords: Depression in children, Parental loss, Peer bullying

INTRODUCTION

Depression in school-aged children and adolescents is a growing public health concern. Among the most consistently identified psychosocial risk factors are parental loss (particularly death of a parent) and peer bullying. Both exposures are independently associated with increased risk of depressive symptoms and clinical depressive disorders, and emerging evidence suggests that combined exposure may confer multiplicative risk. Children who experience the death of a parent are at elevated risk of developing depression. Large-scale cohort and registry-based studies show that parental loss in childhood is associated with subsequent major depressive disorder, as well as other psychiatric outcomes (1,2). The risk is influenced by age at loss, sex, and family psychiatric history. Bereaved children often show higher depressive symptom trajectories that may persist into adolescence and early adulthood (3). Bullying victimization is a robust predictor of depression in childhood and adolescence. Meta-analyses consistently show that bullied youth have higher rates of depressive symptoms, with longitudinal data supporting a dose–response relationship (4,5). Both traditional bullying and cyberbullying predict increased depression, with effects persisting into adulthood in some cohorts (6). There is growing evidence that parental bereavement and bullying interact to exacerbate risk. Some recent studies suggest bereaved children may be more vulnerable to peer victimization, and the combined effect of both exposures results in higher depression risk than either alone (7). Several mechanisms have been proposed to explain the link between parental loss, bullying, and depression:

1. Attachment disruption and caregiver stress.
2. Neurobiological dysregulation (HPA-axis, inflammation).
3. Negative cognitive schemas and maladaptive social processing.
4. Social isolation and reduced peer/family support.
5. Cumulative stress and socioeconomic strain.

The impact of loss and bullying varies depending on developmental stage, sex, socioeconomic status, and cultural context. Girls in adolescence often show higher rates of depression following both exposures. Early childhood exposure may predict chronic trajectories, while adolescence marks a sensitive period for onset (8). Parental loss is typically documented through caregiver reports or administrative data, while bullying is usually self-reported. Multi-informant approaches improve validity. Outcomes are measured with structured diagnostic interviews or

validated scales (e.g., CDI, PHQ-A). Longitudinal studies provide stronger causal inference, but many studies remain cross-sectional (9). Here are summary of studies showing potential causes of depression in children.

Study	Sample	Exposure	Main Findings
Li et al. 2022 (1)	Population registry (N>1M)	Parental death	Higher risk of major depression in bereaved children
Melhem et al. 2008 (3)	Community cohort	Parental death	Increased depressive symptoms, persisting into adolescence
Ye et al. 2023 (4)	Meta-analysis	Bullying	Victimization strongly associated with depression
Tong et al. 2024 (5)	Longitudinal studies (meta-analysis)	Bullying victimization	Predicts later depressive symptoms
Boelen et al. 2021 (10)	CBT Grief-Help	Bereaved children/adolescents	Reduced prolonged grief & depression
Fraguas et al. 2020 (11)	Anti-bullying programs	School populations	Reduced bullying, improved MH outcomes

All the reported studies have shown parental loss and bullying as significant factors causing depression. However, there was limited literature from Pakistan. Thus, this study was designed to evaluate parental loss and bullying as factors causing depression in school children.

METHODS:

This was a descriptive cross-sectional study conducted on school children of rural and urban schools of Tando Muhammad Khan district. A total of 1000 students were included, and a predesigned questionnaire was distributed to get information. Assistant teachers helped gather responses from the children. The questionnaire was adopted from Patient Health Questionnaire – 9 (PHQ-9) Modified PHQ-A, was used for data collection. It has nine questions and scored from 0-3 for four responses including not at all to nearly everyday respectively. The responses were summed up at the end to make a score of the depression. The score was categorized as given below:

0–4: Minimal or none
5–9: Mild
10–14: Moderate
15–19: Moderately severe
20–27: Severe

Statistical analyses

The data was analysed by using Statistical Package for Social Sciences (SPSS software version 22.0). Descriptive statistics are presented as number and percentage. Chi-square test was used for analysis of categorical variables and a p-value <0.05 was considered statistically significant.

RESULTS:

A total of 1000 students were included in this study, median age of the participants was 9 years (±SD= 2.82, range 5 - 15). There were 517 (51.7%) males and 483 (48.3%) female students. 946 students living with two parent household, while 11 students lived with single parent following divorce. A total of 38 students were living with single parent after death of one parent and 5 students lived with extended household after death of both parents. A total of 53 students had a history of bullying out of which 13 lived in two parent household. Majority of students had 1-2 hours screen time with smart phone while 158 students reported no time with screen. A total of 43 students reported no participation in extracurricular activities. No depression was reported in 939 (93.9%) students, pattern of mild to severe depression is presented in Figure 1. The rate depression was significantly associated with history of bullying regardless of their household pattern (0-value <0.001). Those showed severe depression 50% (n= 3) had history of bullying. Among family pattern the rate moderately severe and severe depression was reported in students with single parent where the high rate was seen in those death of one parent. The rate of depression among single parent family was not associated with screen time. While depression score was significantly associated with no participation in extracurricular activities. Where 66% of severe depression score students denied taking part in extracurricular activities (p-value <0.001).

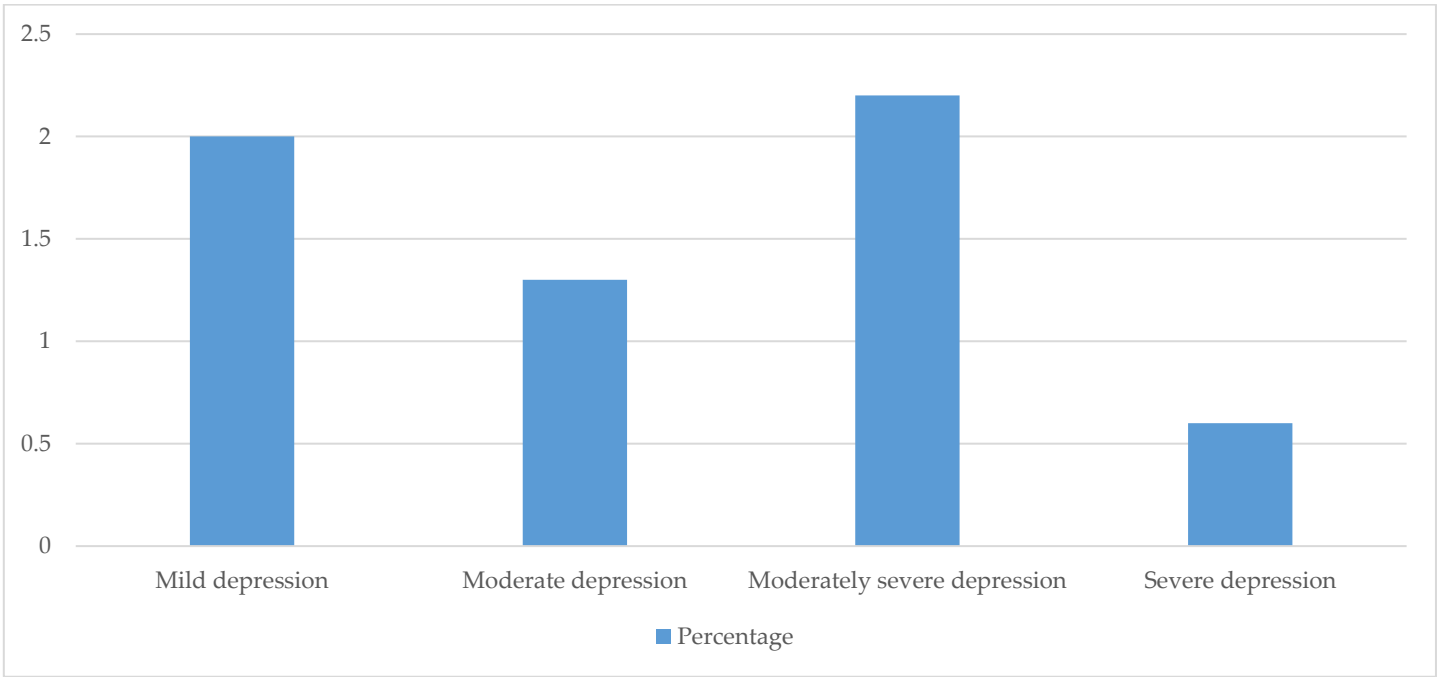

Figure 1. Pattern of severity of depression in school children

DISCUSSION

The present study investigated depression among school-going children aged 5–15 years, with particular focus on family structure, bullying, screen time, and extracurricular participation. The median age was 9 years and the sample was nearly gender-balanced. Overall, 6.1% of children reported varying degrees of depression, consistent with global estimates of childhood depression ranging between 2% and 8% in community-based samples (2,3). A key finding was the significant association between bullying and depression, irrespective of household pattern. Half of the students with severe depression reported a history of bullying, highlighting the substantial psychological burden of peer victimisation. This is consistent with large meta-analyses, which confirmed that bullying victimization is strongly associated with subsequent depressive symptoms and clinical depression, often in a dose–response manner (1). Importantly, our findings reinforce that the negative mental health impact of bullying transcends family background, underscoring the critical role of peer interactions in child well-being. Family structure also emerged as a relevant factor. While the majority of children resided in two-parent households, students from single-parent families following the death of a parent had higher rates of moderate to severe depression compared to peers in intact families. This aligns with prior longitudinal studies demonstrating that parental bereavement increases the risk of depression, anxiety, and other psychiatric disorders in childhood and adolescence (6-8). The heightened risk may reflect disruption in attachment, reduced social support, and increased caregiver stress after bereavement (7). Notably, single-parent families due to divorce showed lower depression prevalence than those due to parental death, suggesting the permanence and emotional weight of death may exert a more profound psychological toll (5).

Interestingly, depression scores were not significantly associated with screen time. This contrasts with some studies linking excessive screen use to poor mental health outcomes in children (11-13). However, our sample reported relatively modest screen exposure (1–2 hours daily), a level that is within the range often considered non-problematic (5). These findings suggest that moderate screen time may not independently predict depression in this age group, though its interaction with other psychosocial stressors warrants further investigation.

By contrast, non-participation in extracurricular activities was significantly associated with depression. Two-thirds of students with severe depression reported no engagement in extracurricular activities, indicating the protective role of structured, prosocial engagement. Prior literature supports that extracurricular involvement fosters resilience, social connectedness, and emotional regulation, thereby reducing depression risk (4-6). Our findings highlight the importance of encouraging children's participation in such activities as a preventive strategy.

Taken together, this study emphasizes that bullying victimization and parental loss are major psychosocial determinants of childhood depression, while extracurricular participation appears to offer a protective buffer. These findings have important implications for schools and policymakers. Anti-bullying interventions should be prioritized as universal prevention strategies, while targeted psychological support should be made available for bereaved children. Additionally, efforts to increase opportunities for extracurricular engagement may help promote resilience in vulnerable populations. This study had some limitations. The cross-sectional design prevents causal inference, and depression was assessed using screening rather than diagnostic interviews, which may underestimate subclinical presentations. The small number of children in single-parent families limited statistical power for subgroup analysis.

Nonetheless, the findings contribute valuable insights into the intersection of family structure, peer victimization, and child well-being in our context.

CONCLUSION

Our study demonstrates that depression in school children is strongly associated with bullying and parental loss, with extracurricular participation offering potential protection. Early identification and school-based preventive programs are critical to mitigating these risks and promoting mental health among children.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by local research ethics committee.

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Case Report

A RARE PHENOMENON OF ANTI-N ANTIBODY REACTIVITY AT 37°C: AN UNCOMMON DETECTION IN ROUTINE IMMUNOLOGICAL SCREENING

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DOI: 10.38106/LMRJ.2025.7.2-07
Received: 16.04.2025
Accepted: 10.06.2025
Published: 30.06.2025

ABSTRACT:

Anti-N antibodies usually possess cold-reactive properties which make them clinically insignificant and are mostly classified as naturally occurring IgM antibodies. These antibodies sometimes trigger reactions at body temperature (37°C) or in the anti-human globulin phase resulting in serious medical outcomes such as delayed hemolytic transfusion reactions or hemolytic disease of the newborn. This case shows an extraordinary naturally occurring anti-N antibody which was detected in a 20-year-old male liver donor during standard antibody testing because of its unexpected reactivity at 37°C. The absence of any previous blood transfusions or medication use in the patient made this case stand out as particularly intriguing. The antibody identification was successful following the 'pre-warm' technique implementation while antigen phenotyping validated it as an IgG-type anti-N antibody. This medical case demonstrates the critical importance of recognizing unusual antibody responses during blood transfusions and organ transplants because uncommon reactions may lead to serious consequences.

Keywords: Anti-N antibody, MNS blood group system, Transfusion medicine, Serological testing, Delayed hemolytic transfusion reactions

INTRODUCTION

The MNS blood group system, discovered by Landsteiner and Levine in 1927, was the second to be identified after the ABO system. Among the antibodies in the MNS system, anti-M is a common "naturally occurring" antibody (1). The S antigen was identified in 1947 by Walsh and Montgomery after the development of the antiglobulin test (2). Most anti-M antibodies are cold-reactive and do not activate complement or react with enzyme-treated RBCs (3). They are rarely associated with hemolytic transfusion reactions (4).

Anti-N antibodies are less common than anti-M and are also typically naturally occurring, cold-reactive IgM or IgG agglutinins that do not activate complement or react with enzyme-treated RBCs (1). They are clinically insignificant unless reactive at 37°C and have been linked to rare cases of mild hemolytic disease of the fetus and newborn (HDFN) (5). Potent anti-N antibodies are more frequently found in individuals of African descent with a specific RBC phenotype (M+ N- S- s-) due to the absence of the N antigen (2). Immune anti-N antibodies are extremely rare (6). We report a case of naturally occurring anti-N that reacts at 37°C, identified during routine antibody identification testing (7).

CASE REPORT

A 20-year-old male from Karachi, who's healthy and without any remarkable medical history, decided to donate liver to his father. His father had been struggling with chronic liver disease because of a hepatitis B infection, so they set up a liver transplant at Dow University of Health Sciences, Karachi, Pakistan. The donor had never had a blood transfusion or taken any medication. For the usual pre-transplant checks, they sent a test request to Aga Khan University Hospital to identify any antibodies. The first blood tests came back showing a positive auto-control, which means his red blood cells reacted with his own serum at room temperature. However, when a Direct Antiglobulin Test (DAT) with anti-IgG + C3d, was performed it came up negative, ruling out any autoantibodies. Then three different antibody screening panels (ID-Diacell I-II-III, Biorad) were checked and found positive reactions in all of them (2+, 3+, and 3+). It was also noticed that the auto-control was positive right from the spin phase. So, it was decided to run the antibody screening again using a method where the sample was warmed everything up and the results were positive across all three panels. In order to further explore the Papain treatment

method (ID-Diacell Papain Kit), was used which came up negative for the red cell antibody screening. But when further evaluation was performed with the antibody identification using the warm technique, it was found that he had an anti-N antibody using this 11-cell identification panel (ID-Diacell, Biorad). The reaction was strong 3+, with homozygous N+ N+ cells (Panels 4, 10, and 11) and negative with heterozygous M+ N+ cells (Panels 1, 3, 7, and 8), plus also negative with N-negative cells (Panels 2, 5, 6, and 9). Then other antigens for N, S, s, and M were also checked and found that he was M-, N-, S-, and s-. Then his plasma was treated with dithiothreitol, which confirmed there was an IgG-type anti-N antibody present. The antibody titer was 1:2. This report emphasizes a naturally occurring anti-N antibody that reacts at 37°C, which could actually be important even though the donor hadn't had any blood transfusions before. It is therefore, highlights the need to check for these naturally happening antibodies during routine blood tests because their reactions at body temperature can affect blood transfusion practices.

DISCUSSION

Anti-N antibodies belong to the MNS blood group system and are usually naturally occurring and mainly cold-reactive IgM antibodies. These antibodies are often clinically insignificant unless they decide to react at 37°C or during the anti-human globulin (AHG) phase of testing. In this case, we found the anti-N antibody in a 20-year-old male liver donor who had a serological profile showing an uncommon blood group discrepancy. While anti-N antibodies are generally more of a cold-reactive type and don't usually bind complement, if they do react at 37°C, that's when raise the concern about their clinical significance.

Usually, anti-N antibodies do not cause major issues, as they are linked to non-pathological clinical outcomes. They typically do not lead to hemolytic transfusion reactions (HTRs) or hemolytic disease of the fetus and newborn (HDFN) unless they react at body temperature (37°C), like in this case (1). A transfusion reaction is more likely if these antibodies show strong reactivity at 37°C, but that's pretty rare. When it occurs, it can lead to delayed hemolytic reactions, which may result in certain transfusion-associated complications. Besides, while HDFN can be a concern in cases of maternal-fetal blood group incompatibility, instances of HDFN linked to anti-N are sporadic (2).

Interestingly, the IgM class of anti-N antibodies usually reacts in colder conditions and doesn't typically bind to complement or react with enzyme-treated red blood cells (RBCs). This is kind of similar to anti-M antibodies, which show the same cold-reactive behaviour and limited clinical relevance, unless they react at body temperature (3). However, in our case, the antibody was behaving unusually by reacting at 37°C, warning for atypical antibody behaviors in blood donors, especially when it comes to organ transplant recipients who might need careful cross matching and serologic evaluations.

The phenotyping results were non-significant since the donor's red cell antigen profile showed the absence of the M, N, S, and s antigens, which are usually part of testing in the MNS blood group system. In addition, after treating with Dithiothreitol (DTT), it was confirmed that the anti-N antibody was of the IgG nature, suggesting that it could have some clinical significance in certain transfusion scenarios (4).

Finding an anti-N antibody in a healthy individual with no prior blood transfusions is unusual, given that naturally occurring antibodies in the MNS system are typically IgM types and do not usually trigger immune responses. The titer of 1:2 suggests the anti-N antibody could be clinically important at 37°C (7). This goes to show how important it is to do a thorough antibody screening for blood donors; even naturally occurring antibodies might need attention in a clinical setting.

CONCLUSION

In conclusion this case really emphasizes the importance of rare blood group antibodies in organ transplantation and transfusion medicine. It's a reminder for clinicians and lab staff to keep a closer look at unusual antibody profiles, especially when there are discrepancies in routine serological testing. We need more studies and awareness about these antibodies to avoid adverse reactions in transfusions and organ transplant procedures.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

Informed consent of the patient was taken.

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