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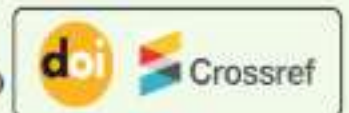


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The Dawn of Molecular Medicine: Revolution in Healthcare

Talat Mirza

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The era of personalized medicine has begun as the healthcare ecosystem moves from a one-size-fits-all approach to tailored molecular targets. This discovery ushered in the field of molecular medicine, which investigates the complex molecular genetic events implicated in health and disease. It relates to the interaction of genes, proteins, and Biomolecules affecting the pathophysiological pathways, paving the way for personalized therapies and targeted diagnostics.

The modern terrain of medicine is changing by one of the magic wands of molecular science. Recent studies exhibit valuable contributions in the incorporation of molecular pathology with diagnostics and therapeutic influences revolutionizing clinical practice with research-mapping directing a new course towards precision healthcare¹. Molecular pathology has been one of the corner stones in this transformation. It makes dissection of tissue/cellular alterations at the genetic and molecular levels for more accurate diagnosis, better prognostic assessment, and directed treatment strategies. Therefore, translational medicine forms a bridge between the basic biomedical research and clinical practice as a bench to bed side and bench to industrial approach².

The remarkable ability to identify biomarkers both on a genetic and molecular scale from patient samples enables early intervention and offers more precise molecular characterization, most relevant in Oncology and Infectious diseases³. The other important emerging area that promises transformation is targeted therapeutics and personalized interventions at individual molecular, genetic levels. Personalized treatment regimen seems increasingly attainable and holds promise even for conditions previously thought to be incurable like certain cancers, autoimmune disorders, and complicated infectious diseases².

Thus, the convergence of these new avenues creates a future for tomorrow's medicine not simply creative

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but predictive and tailored for individualized care. The recent advances in technologies like AI and machine learning, have augmented with unprecedented tools to enhance disease characterization with improved prognostic models, and personalized treatment approaches enhancing the accuracy and customized plan⁴.

This holistic, molecular-based application marks a turning point in healthcare ushering in an era where the complexity of disease is met with highly sophisticated, individualized solutions. Molecular medicine is not only a theoretical concept; it has unquestionable practical implications in the modern era. Early and precise diagnosis, frequently before symptoms appear which relates to finding disease biomarkers. The use of molecular profiling of tumors, now the best therapy for cancer patients can be based on their unique genetic makeup. In sharp contrast to the conventional, less focused and more generic methods, this accuracy reduces side effects and maximizes therapeutic efficacy. Furthermore, drug designing and development is being totally revolutionized by the emergence of customized pharmacogenomics, which predicts drug reactions based on an individual's specific genetic constitution⁵.

Complex diseases like metabolic syndrome, neurodegenerative disease, cardiovascular disease and understanding of the molecular details is crucial for devising new therapeutic strategies⁶. The strength of molecular medicine is demonstrated by recent groundbreaking studies denoting mutations that cause disease through CRISPR-Cas9 gene editing technology opening up hitherto unheard-of possibilities for treating genetic disorders⁷. Likewise, the discovery of particular microRNAs as biomarkers for a number of illnesses, has yielded fresh perspectives on the etiology of illnesses as well as possible diagnostic instruments⁸. Several remarkable clinical discoveries are based on immunotherapy research notable as CTLA4 and PDL1 discoveries⁹.

Recent advances in molecular medicine have transformed the understanding of diseases and treatment landscape using patients' unique profiles. Some key developments include **CAR-T cell Therapy** which uses genetically modified T cells and **Epigenetic Drugs**

altering gene expression to kill cancer cells; **Adeno-associated viral vectors (AAV)** for gene delivery to ultimately design vectors with enhanced safety; **Liquid Biopsies** relates to the analysis of circulating tumor DNA or RNA for non-invasive cancer detection and monitoring. This entails integrating genomic data with electronic health records and machine learning algorithms for personalized treatment alternatives; **Fast Molecular Tests** for the rapid identification and detection of pathogens for drug resistance. This movement has been spearheading changes toward patient's unique profile, allowing healthcare professionals to be well acquainted with solutions and more efficacious treatments.

Molecular medicine relies fundamentally upon omics technology for advancing the translational research in medicine including transcriptomics, metabolomics, proteomics as well as genomics¹⁰. The combination of metabolomics with artificial intelligence (AI) has become a game changer in precision oncology. According to Chen et al., artificial intelligence (AI) streamlines data collection and processing to interpret intricate metabolic pathways enabling more successful multiomics integration and biological data analysis^{11,12}.

The advancement of molecular medicine is strongly supported by the incorporation of artificial intelligence (AI). Large-scale molecular data sets, such as proteomic, metabolomic, and genomic data, can be analyzed by AI algorithms to find trends and forecast treatment outcomes, medication response, and disease risk. Machine learning models also contribute to new drug design by identifying promising therapeutic targets or predicting molecular interactions¹³.

The greatest avenue of advancement for personalized health is in the ways that artificial intelligence and molecular medicine may develop into combining new predictive, preventative, and precision medicine strategies. In fact, advancements in AI have greatly enhanced Digital Nucleic Acid Amplification Test (dNAAT), thus improving high throughput analysis for improved accuracy and efficiency of molecular diagnostics. Such integration will pave the way for more point-of-care testing systems that were previously prevented from development due to cost, complexity, and data interpretation software.

Ziauddin University is the first university in Pakistan that offers specialized College of Molecular Medicine in the field providing degree programmes ranging from **bachelor's (BS)**, **master's (MPhil.)**, and **doctoral (PhD.)** degrees in the area. This effort aligns with the university's vision of changing the face of healthcare with innovative teaching and research. Ziauddin University proudly promotes the training of physician scientists with bench to bedside approach creating a new culture of scientific inquiry in health research. We, at Ziauddin University, stand at the doorstep of a new age of precision medicine, unravelling disease

complexities and their solutions in healthcare; hence it is time to invest in and heighten awareness about molecular medicine.

In conclusion, molecular medicine is not merely a new emerging scientific discipline; it is the very foundation of future healthcare or better be called as 'tomorrow's medicine'. The time may come when diseases will be diagnosed earlier, treated more successfully, and perhaps even prevented with the adoption of its tenets, with the use of modern technologies based on AI and Omics.

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Assessment of Sources of Academic Stress and Coping Strategies Among Junior Dental Students of Preclinical Years in A Public Sector University

Hina Shah¹, Sanaa Ahmed², Syed Moosa Raza³, Mariam Irshad⁴, Maham Ahsan⁵, and Zainab Saifuddin⁵

ABSTRACT

Objectives: To identify the sources of stress and coping strategies among preclinical students and to compare dental environment stress, the self-perceived stress, and coping strategies scores

Methodology: A cross-sectional study was conducted in a public sector dental school of Karachi on the students of the first and the second year. Students were asked to fill in the questionnaire based on DES, PSS, and COPE scale. Sample size of 89 was calculated through OpenEpi and convenience sampling was utilized for data collection. A pilot study to validate the questionnaire was completed followed by final data collection.

Results: Data was analyzed through SPSS version 18. The number of female participants was 64 out of 89 while males were 25. The first year students were (N=44) and the rest were second year students (N=45). The Mean score for PSS for the first year was 28.06 while for the second year was 28.1. DES showed that the highest stress factors were 'Examination', 'Workload' and 'Performance Pressure' while the highest scoring COPE factors were 'religious coping', 'planning' and 'restraining thoughts'. The overall stress was higher in the second year students than in the first year students with both seeking solace in religious teachings the most. Also, female participants showed high stress scores.

Conclusion: The findings of the study support previous data that dental students suffer from constant stress and pressure to perform which may lead to mental issues and eventually burnout. Support by teachers and family could play a major part in managing their stress levels.

Key Words: Academic Success Motivation, Quality of Life, Universities, Workload

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INTRODUCTION

Stress is a commonly encountered factor that affects the mental health of students and individuals alike. Defined as any change that affects the functional ability

of a person including physical, emotional, or psychological strain. It can be an intrinsic or extrinsic factor invoking a biological response¹. In certain individuals, stress can be a motivating force but in excess it can affect the mental health negatively². It generally exceeds the capacity of an individual to respond and can potentially have adverse physical, psychological, and emotional consequences. It can make a person feel overwhelmed, fatigued, nervous, and miserable. Stress may induce changes in eating and sleeping habits, cause headaches, and may result in loss or gain of weight. In the long run, stress can exacerbate the risk of depression, anxiety, heart disease, and diabetes^{3,4}.

The variables invoking stress response of the body resulting in anxiety, tension, or fear are termed as stressors. Internal stressors include continuous thinking or certain behaviours. Usually, these thoughts originate from high expectations or one's psychological mindset, for instance, fear of heights and spiders. Other stressors

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are external factors, originating outside one's mind. These are forces that you cannot easily control. Examples include major life events, urgent deadlines, or exams^{1,5}.

Continuous high stress levels may affect focus, short- and long-term memory and decision-making capacity. These outcomes appear to be governed by the individual's assessment of the requisites and resources of a situation, the relationship between the stressor and the task, and aspects such as coping styles, locus of control, and social support⁵.

Stress can have a negative impact on physical well-being, mental health, and academic success unless it is managed aptly. Students entering higher studies frequently express that they feel stressed due to changes in living conditions, workload, responsibilities, and peer interaction. Intense stress can affect the functional capacity resulting in decrease in work effectiveness and poor academic performance. Dental school environment is highly competitive and intensive due to constant pressure to meet deadlines and maintain grades causing psychological distress to students and making them prone to anxiety and depression compared to other disciplines^{1,3,5}.

Dentistry is an exceedingly stressful profession, starting as a student and progressing into clinical practice. Elevated stress levels may have a negative influence on dental students' learning ability. Students predisposed to stressful life events also report reduced quality of life. Managing stress for dental students is crucial or else prevailing stress may lead to destructive outcomes including depression, dropping out, drug abuse, and suicidal thoughts².

Introducing successful coping strategies may help students avoid the destructive consequences of excessive stress^{5,6}. Many studies worldwide have examined perceived stress among dental students. However, our study specifically aims to assess perceived stress levels, identify key stressors, and evaluate coping mechanisms among junior dental students at a public dental school in Pakistan.

METHODOLOGY

IRB/ERC Approval:

A cross-sectional study was conducted at the Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University during the month of October 2022. Permission from the Institutional Review Board of JSMU (Ref: NO: JSMU/IRB/2022/675) was taken prior to the data collection.

All junior dental students of preclinical years (1st and 2nd year Bachelors of Dental Surgery), above 18 years of age, regardless of gender, religion, caste, creed, and socio-economic backgrounds were included in the study. While those who did not consent to participate or submitted incomplete forms were removed from the study. Sample size was calculated to be 89 out of 102 total students at 99% confidence interval through OpenEpi 3.01. The sampling technique utilized was non-randomized convenience sampling. Students were approached in their respective classes and were informed about the study objectives and aims. They were asked to participate and those who agreed were given the questionnaire. Forty-five students from the 1st year and 44 students from the 2nd year participated in the study.

We used a combination of three structured validated questionnaires 'Dental Environment Stress', 'Perceived Stress Scale', and 'COPE' to collect data. The questionnaires consisted of close-ended questions.

DES questionnaire was developed by Garbee *et al.* It appraises stressors in an undergraduate dental student's life related to academic performance, clinical practice, faculty relations, personal life issues, professional identity, and financial obligations. All the above items were evaluated except those pertaining to clinical exposure in this questionnaire because the sample included only the first- and second-year students. All participants ticked the most suitable option available with the item on a 4-point Likert scale from 'not stressful at all' to 'very stressful'. Sum of all responses was given a final score⁶.

The Perceived Stress Scale (PSS) measures the degree of stress perception⁷. The questions in the PSS evaluated feelings and thoughts during the previous month by asking the respondents to tell how often they encountered stress in a certain situation by choosing their answer from 5-point Likert scale from 'Never=0' to 'Very Often= 4'. Score was the sum of all answers. COPE Inventory: Fifteen items were selected out of 60 from the inventory and rephrased for each coping category that can be utilized by students in response to their stress. The options include 'I have not been doing this at all=1' to 'I have been doing this a lot=4' on a 4-point Likert scale, were recorded¹.

The data collected was analyzed and entered using the software IBM SPSS statistics version 26. The collected data was organized as descriptive results, and included the student's age, gender, and year of study; then these were analyzed and tabulated as frequency and percentage distribution. The mean score and SD for each of the measures under research was tabulated.

Statistical analysis was done after dividing the questionnaire into three categories: thirty stress-related items, ten-item PSS (along with the reversal of coding to the four positively stated items of 4, 5, 7, and 8), and fifteen items for coping strategies. The Mann-Whitney test was employed to determine significant differences between first- and second-year students. Differences between individual years are going to be assessed using a pairwise comparison test. Pearson's correlation and beta coefficient was calculated to compare the strength of the effect of coping strategies in relation to the perceived stress scale scores. The statistical significance (p-Value) is to be set below 0.05 (95% confidence interval).

RESULTS

Students from first- and second-year of dental school were approached to take part in the study. A total of 89 pro formas were received after filing. Out of those, 45 were from first-year students and 44 were from second-year students. The male to female ratio was 2.5 with 25 males and 64 females participating in the study. Pilot study on 10 students was done to check for the face validity of the DES, PSS, and COPE scales as we had modified the questionnaires and found them to have good face validity while the Cronh Bach's Alpha was calculated for each questionnaire separately and found to be 0.7 for PSS and COPE scale, while for DES scale it was 0.814, all showing good reliability. The data of the pilot study was included in the results.

Table 1 shows the perceived stress levels of the students from both years and also according to the gender. We found that there is only slight difference in the stress levels among the genders with the males scoring less than females. No difference was found between the scores of first-year and second-year students. Both showed high stress levels. The Mann-Whitney U test comparing perceived stress levels between 1st-year and 2nd-year BDS students yielded a U-value of 1167 which is significant to the level of 0.05 showing that there is no strong evidence of a significant difference in perceived stress levels between the compared groups.

Table 1: Self-Perceived Stress Scores According to Gender and Academic Years

S.No.	Variable	N	Mean	(S/D)	
1.	Gender	Male	25	26.8	6
		Female	64	28.6	5.6
2.	Year of Study	First Year	44	28.06	6.1
		Second Year	45	28.1	5.2
3.	Mann-Whitney U (Academic Years)	89	1167 (sig.=0.145)	1167 (sig.=0.145)	

Table 2 shows the different factors causing stress in students. The mean scores showed that the highest stress was from examination (mean score:2.89±0.5) followed by workload (mean score 2.74±0.63) and performance pressure (mean score 2.59±0.67). These scores correspond to the quite stressful category of the questionnaire.

Table 2: Stress Causing Factors from Dental School / Environment During Academic Years

S.No.	Factor	(N)	DES Mean Score (MS)	(S/D)
1.	Examination	89	2.89	0.50
2.	Workload	89	2.74	0.63
3.	Performance Pressure	89	2.59	0.67
4.	Faculty and administration	89	2.53	0.68
5.	Self-efficacy	89	2.41	0.52
6.	Peer interaction	89	1.83	0.68

Table 3 shows the coping strategies utilized by the students to cope with stress. The most commonly utilized strategy was religious coping strategies (mean score= 3.11±0.92), followed by planning activities to achieve desired scores (mean score= 2.9±0.90), and restraining negative thoughts (mean score= 2.74±0.936).

Table 4 shows the difference between the scoring among the two academic years on DES, PSS, and COPE scale. The items showing significant differences at the level of 0.05 (marked by*) and 0.001 (marked by**) are summarized. Item 2 and 5 from DES scale showed high significance (p= 0.001) while item 1,3,4,6 on DES and item 1, 2, and 3 from PSS showed significance of up to 0.05. No items from COPE scale showed any significant difference. All the items summarized were scored higher by the second-year students compared to the first-year students.

DISCUSSION

Dental students are under multiple kinds of stress during their academic life including financial and health related issues. Several studies have examined the stress and coping strategies of students across the academic years including their clinical training years^{1-4,6-9}. For this purpose, the widely utilized tools are DES for dental environment related stress factors, PSS for stress levels, and the COPE inventory. Our objective was to find the sources of stress from the academics; hence we included only students from the first and second year of Bachelor of Dental Surgery in our sample.

Table 3: Coping Strategies Utilized by Students

S No.	Item	Mean Score	(S/D)
1.	Implementing religious coping in my lifestyle	3.11	0.922
2.	Planning activities and prioritizing tasks	2.9	0.905
3.	Trying to restrain my thoughts	2.74	0.936
4.	Bringing humor into play as a psycho-effective technique	2.73	1.074
5.	Trying mental disengagement and diverting my mind	2.73	0.951
6.	Practicing behavioral disengagement and self-isolation	2.72	0.879
7.	Focusing on priorities and venting of emotions	2.71	0.968
8.	Practicing positive reinterpretation and growth	2.62	0.911
9.	Choosing to use the strategy of denial	2.48	0.955
10.	Employing acceptance as a behavioral coping strategy	2.48	0.854
11.	Attempting to suppress the idea of competing activities	2.45	0.905
12.	Seeking active coping	2.38	0.846
13.	Relying on substance use for distraction	2.37	0.958
14.	Making use of emotional social support	2.35	0.966
15.	Making use of instrumental social support	2.3	1.060

Table 4: Comparison of Scores Between Two Academic Years on DES, PSS, and COPE Scales

Scale	Items	Academic Year	N	Mean	SD	Sig.
DES Scale	1. Having a lecture or laboratory session immediately before my exam	1	45	2.78	1.185	0.045*
		2	44	3.25	.991	
	2. Difficulty of course work	1	45	2.62	.684	0.011*
		2	44	3.02	.762	
	3. Inadequate breaks between lectures/sessions	1	45	2.00	.953	0.030*
		2	44	2.50	1.171	
	4. Criticism about my academic performance	1	45	2.13	1.198	0.022*
		2	44	2.68	1.006	
	5. My dependencies (smoking or medication)	1	45	1.20	.588	0.008*
		2	44	1.70	1.091	
	6. Rules and regulations that I am required to follow in dental school	1	45	1.89	.982	0.010*
		2	44	2.50	1.191	
PSS Scale	1. I feel left out when my peer group discusses learnt concepts in their native languages	1	45	1.98	1.252	0.02*
		2	44	2.59	1.187	
	2. Focusing on priorities and venting of emotions	1	45	2.47	1.014	0.017*
		2	44	2.95	.861	
	3. Employing acceptance as behavioural coping strategy	1	45	2.27	.863	.015*
		2	44	2.70	.795	

Significance value marked with * are significant up to the 0.05

Since we rephrased and selected items from each questionnaire according to our need, a pilot study was conducted to validate and find out the reliability before final data collection. Once the face validity and Cronh Bach`s Alpha was calculated, questionnaires were distributed among the students. Total 89 students participated out of whom 25 were males and 64 were females. The PSS score for females was higher than

their male counterparts which is similar to the results from other studies showing gender predilection to stress^{8,9}. The PSS scores of the first-year and second-year students were similar responding to high stress levels. However, comparing these to results from studies on students of Ajman, showed higher scores in first-year than in second-year students¹⁰.

The stressors in our study were examination (MS=2.89), workload (MS=2.74) and performance pressure (MS=2.59). On comparing these top three stressors with those in the study conducted in Ajman, we found that the results were similar to ours that is the 'amount of study load' (64%), 'fear of not being able to catch up if falling behind' (60%) and 'examinations' (59.5%). While the study from India reported that their students scored 'vastness of academic curriculum' (47.7%) as the highest stressor followed by 'fear of failure or poor performance' (34.2%) and 'lack of recreation' (12.6%). All three studies supported that a major part of the stress among the dental students was from academic load and examination⁸⁻¹⁰.

The most common coping strategies utilized by our sample were 'religious coping', 'planning' followed by 'restraining thoughts', comparable to the findings from Ajman, and Poland with the highest sought strategy as 'religious coping'. It was alarming that a small percentage of students used 'substance' consumption to cope with stress. This emphasized that providing students with counselling services is necessary and the amount of workload should be decreased and distributed across the calendar year. Parents of susceptible students should be brought on board and counselled to decrease other manageable factors such as discussing their expectations or setting them too high for the individual to achieve. Stress is frequently linked to anxiety and depression that may lead to suicidal thoughts and susceptibility to follow them¹¹⁻¹⁴.

The scoring for all three scales among the two academic years showed significance in DES and PSS items. Second-year students mostly showed significantly higher scores for workload related items compared to first-year students on DES scale, while on PSS scale, second-year students scored higher than the first-years on 'I feel left out when my peer group discusses learnt concepts in their native languages', 'focusing on priorities and venting of emotions' and 'Employing acceptance as behavioral coping strategy'. No significant differences were found in COPE inventory. In comparing them to results from Ajman, 'mental disengagement', 'venting of emotions' and 'active coping' showed significant values⁸. Furthermore, when these were assessed with findings from Poland, significant differences showed between PSS and coping strategies between the first- and second-year students for the items 'venting of emotions', 'substance use', and 'suppression of activities'¹¹.

The findings of our study are supported by previous studies conducted on the factors affecting the stress levels in dental school environment and academic

workload. All studies are in support that high level of stress is the major reason behind anxiety and depression and high burnout of not only the undergraduate dental students but also the postgraduate students and practitioners alike,^{1,6,12,13,15-18}. The policy makers and curriculum designers should address this issue and provide support to students to improve the quality of education provided and to produce skilled motivated clinicians.

The current study has its own limitations including a small sample size and data from a public sector university which could affect the generalizability of results. Also, the environment and stress factors of private institutes may differ and hence the stress levels and stressors as well as the coping strategies. Hence, a multicenter study design with large sample size is required to offer a more realistic picture.

CONCLUSION

The burnout rate in dental practitioners is high across the globe. The main reason behind is the stressful dental environment causing anxiety and depression. To help the students in managing stress during their academic years, multiple steps are required including reforms in curriculum, counselling sessions along with extracurricular activities and workshops to manage stress. All this will help to provide a healthy learning environment to improve the quality of the practitioners produced and decrease the burnout rate in dentists.

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Application of Quality Control Parameters and Model Dependent and Independent Approaches on Different Brands of Itopride HCL 250mg Available in Karachi, Pakistan

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ABSTRACT

Objective: To conduct Invitro Quality testing of five different brands Itopride HCl (250mg) available in Karachi, Pakistan to ensure that their quality meet the desired compendial standards.

Methodology: Several In-vitro tests were executed on five different brands of Itopride HCl coded as 11, 12, 13, 14 and 15. Sample of 20 tablets from individual selected brands were subjected to different tests including weight variation, hardness, thickness and diameter. Multiple point intervals dissolution were performed in 0.1N HCl medium in USP paddle type II apparatus and % dissolution data were subjected to several kinetic model including model dependent and model independent approaches utilizing DD solver add in program in Microsoft Excel.

Result: Weight variation of all five coded brands of Itopride HCl 250 mg were found to be 133- 311mg. The disintegration time of all test formulation was between 2 minutes 40 seconds and 9minutes 32 seconds, % friability of all tested tablets was found to be 0.21-0.57%. Multiple point dissolution studies samples were taken at 5, 10, 15, 20, 25 and 30 minutes and drug released was analyzed on UV spectrophotometer at the wavelength of 258nm. Similarity factor (*f1*) considering I2 as reference formulation were found to be in the range of 1.86-6.52 and dissimilarity factor (*f2*) values were found to be in the range of 61.80-84.87. Kinetic models were successfully applied to the dissolution profile of Itopride HCl.

Conclusion: Evaluation of the quality attributes of five different selected brands of itopride 250 mg tablets in Karachi, Pakistan, specifically assessing weight variation, hardness, thickness, diameter, dissolution, and disintegration was the primary objective of this study. By adhering to established Pharmacopeial standards ensure the product's quality, efficacy, and safety. Tablets ability to release active pharmaceutical ingredient in a timely manner improving patient compliance by providing optimum therapeutic activity. Invitro quality standards ensure the products accuracy in terms of weight, potency and performance. The study high light the importance of rigorous quality control in pharmaceutical manufacturing contributing to improved patient outcomes.

Keywords: Itopride HCl, multiple point dissolution, quality evaluation

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INTRODUCTION

Quality, safety and efficacy of any drug must be such that it improves human health and provides required therapeutic effect with no side effects. These standards must be reliable and reproducible and must not vary from batch to batch. The quality of pharmaceutical

products is of major concern in developing countries¹. An increase in number of counterfeit medicines in the market has affected low and middle income countries due to the lack of facilities². All generics drugs that are pharmaceutically bioequivalent must meet the standards of quality, purity, potency and must be identical in all pharmacopeial evaluation tests³. Counterfeit and substandard drugs are major concern for human health as they increases morbidity and mortality rates⁴. The quality of pharmaceutical products must be such that it should be reproducible among batches and free from batch to batch variation⁵. It is the duty of all the pharmaceutical manufactures to check the quality of the product several time during the shelf life of the product⁶. Invitro quality tests or in

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process control of the pharmaceutical products during and after production according to the pharmacopeial test plays a significant role in maintaining the quality standards of the product⁷.

Itopride HCl is the most extensively used prokinetic agent that increases GIT motility by reducing acetyl cholinesterase enzyme, has an anti-Dopamine receptor activity and decreases heart burn, dyspepsia, bloating, nausea and vomiting⁸. Dose of Itopride HCl is 50 mg three times a day with an elimination half-life of 6 hours. It does not cross blood brain barrier and does not impart any CNS effects⁹. The Drug is extensively absorbed and peak plasma concentration occurs in 35 minutes after the oral dosing¹⁰. Itopride HCl belongs to the BCS class I drug¹¹. The present study aim is to perform the Invitro quality testing of several bands of Itopride HCl 250 mg available in Karachi Pakistan to ensure their standards meet the required compendial status.

METHODOLOGY

IRB/ERC Approval:

An exemption letter from the Ethical Review Board was obtained from Nazeer Hussain University, Karachi, under reference number NHU/ORIC/202/202401, dated July 22, 2024.

Five different brands of Itopride Hcl250mg were taken from Karachi, Pakistan's local market and each one was assigned a code for identification i.e. (11, 12, 13, 14 and 15).

Microsoft Excel 2016 software was used for data calculations and for the implementation of kinetic models on multiple point dissolution profile an add-in program DD Solver were utilized, Equipment used in the study included Vernier Caliper (Seiko, China), Friability Tester (Curio FB 2020, Pakistan), Digital Hardness Tester, USP Basket Rack Assembly (DA 6D, Veego, India), USP type 2 Paddle Dissolution Apparatus (Curio, Pakistan), UV Spectrophotometer (Shimadzu, Japan), Analytical balance (Shimadzu, Japan), and distilled water.

This study involved Pharmacopeial tests and non Pharmacopeial tests implementation such as weight variation, friability, disintegration, dissolution, assay, thickness, diameter and hardness. Model dependent and independent techniques were applied for multiple point dissolution. Model independent approaches included difference ($f1$) and similarity ($f2$) factors whereas Model dependent approaches included First-order, Higuichi, Hixson Crowell and Well model. Twenty tablets of Itopride HCl tablets from each

selected five brands were taken and weighed separately. The average weight was then determined. The formula mentioned below can be applied for the estimation of weight variation.

$$\text{Average weight} = (X1 + X2 + X3 + \dots + Xn) / 10$$

$$\text{Weight Variation} = (\text{Individual weight} - \text{average weight}) \times 100 / \text{average weight. (1)}$$

To evaluate the tablets friability or tablets capacity to bear sufficient mechanical strength to withstand mechanical shock during transportation. Roche friabilators were used. Twenty tablets were taken and their weight was noted down collectively (initial weight). The tablets were placed in friabilator and rotated at 100 revolutions for 4 minutes. Once the test was completed, tablets were taken out dedusted, and weighed again (final weight).

The same procedure was repeated for other coded brands. Friability of the tablets were calculated by the following formula

$$\%F = (1 - W/W0) \times 100 \%$$

Friability of tablets <1% were noted as satisfactory.¹²

A Sample of twelve tablets was selected from every coded brands and observed for how well they disintegrated. Tablets of each coded brands were placed in the six tubes of the basket rack assembly of the disintegration tester within an open ended tube on a wire mesh that was fitted at one of its ends. The test consisted of being carried out using distilled water as medium at $37 \pm 2^\circ\text{C}$ for 15 minutes. To prevent the tablets from floating, perforated plastic discs were used. After the complete disintegration of the tablets, the time was noted. According to USP the disintegration time had to be 15 minutes or less. The same procedure was repeated for other coded brands I2, I3, I4 and I5.^{13,14}

To evaluate the thickness and diameter Vernier calipers were utilized, Twenty tablets from all brand were taken and the thickness and diameter was measured individually, by sliding tablets between the jaws of Vernier calipers. Tablet thickness should not vary with in $\pm 5\%$ variation of a standard value.¹⁵ Tests results were evaluated using Microsoft Excel 2019.

Ten tablets from each selected brand were taken and placed in hardness tester. The force needed to crush the tablet was noted in kgF². Average crushing force and SD was calculated using Microsoft Excel 2019. Itopride HCl Spectroscopic method is one of the easiest, the least time and cost consuming method for the determination of Itopride HCl. Take Twenty tablets

were taken and note their average weight then crush the tablets in mortar and pestle and the amount equivalent to 50 mg was taken in 100 ml volumetric flask and diluted with 0.1N HCl. Sample was analyzed in UV spectrophotometer at 258 nm using 0.1N HCl as blank.

The percentage of assay should not be less and more than 90% - 110%.

Dissolution testing was carried out using type II USP dissolution Paddle apparatus at 50 rpm using 0.1N HCl as medium. Samples were taken multiple time as 5, 10, 15, 20, 25 and 30 minutes. Each time 10ml of freshly prepared sample was added after sample withdrawal. By using U.V spectrophotometer at wavelength of 258nm using 0.1N HCl as blank and absorbance of the samples from each point interval were noted and % of drug release was compared with reference¹⁴. Not less than 80% of the drug released at 30 minutes⁸. Dissolution data were subjected to various Model dependent approaches as shown in Table 4.

First order kinetics model^{16,17}

$$\text{Log } Q = \text{Log } Q_0 - \frac{kt}{2.303} \quad (2)$$

Higuchi model⁽¹⁷⁾

$$Q = kt^2 \quad (3)$$

Hixson Crowell model⁽¹⁸⁾

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} \times t \quad (4)$$

Weibull model⁽¹⁹⁾

$$m = 1 - \exp - \left(\frac{t - T_i}{\alpha} \right)^\beta \quad (5)$$

This pair wise method Difference factor (f_1) and similarity factor (f_2) compares the two dissolution profiles such as reference and test formulation^{20,21}. Reference formulation was selected from the test formulations I1-15 on the basis of high drug release I2 was selected as reference formulation as show in Table 3.

$$f_1 = \frac{\sum_{i=1}^n R_i - T_i}{\sum_{i=1}^n R_i} \times 100 \quad (6)$$

$$f_2 = 50 \times \log \left[1 + \frac{1}{N} \sum_{i=1}^n |R_i - T_i|^{2-0.5} \right] \times 100 \quad (7)$$

n = number of samples

R_t = percent release of the reference drug

T_t = percent release of test drug

The value of difference factor (f_1) should be between 0 and 15,

While the Similarity factor (f_2) is between 50 and 100.

DISCUSSION

The aim of the study was to evaluate the different brands of itopride HCl 250 mg available in the market of Karachi, Pakistan. Weight variation indicates the sustainability of good manufacturing practice by the manufacturer as well as the presence of the quantity of an active ingredient in the formulation²². Weight variation tests of all the tablets were found to be in the range of 130.5±6.04-311±15.18 as shown in **Table 1**. Hardness of the tablet indicates the quality of the product. If the tablet is not too hard it will fail in friability test and will be difficult to handle during coating. And if the tablet is too hard, it will not disintegrate in the body which results in poor bioavailability of the product Hardness results of the itopride tablet were found to be between 2.59±0.11 - and 8.5±0.17. shown in **Table 1**. The tendency of the tablet to crumble during manufacturing, handling and transportation can be determined through friability as tablets sliding over one another result in the removal of particles from the surface of the tablet²³. Friability of the test tablets were found to be in the range of 0.29-0.40%. Diameter and thickness plays an important role in terms of tablet swallowing, tablet weight and uniformity of content can be easily detected. Appearance and packing can be affected by the diameter and thickness of the tablet²⁴. The values of diameter found to be in the range of 3.5±0.05- 9.8±0.04 and thickness values were found to be in the range of 2.05±0.03 - 3.3±0.02 as shown in **Table 1**. Disintegration time is considered to be one of the crucial steps for the tablet as it plays an important role in the dissolution of the drug. If the drug does not disintegrate with-in the specified time, it will not be available for dissolution, hence the concentration and efficiency of the disintegrant plays a significant role in the dissolution of the drug²⁵. The disintegration of the test tablets were found in the range of 2 minutes 40 seconds - 9 minutes 32 seconds. Assay indicates the amount of the drug in the tablets and their stability as well²⁶. The Assay of the given the itopride HCl tablets were found to be 100.2 %- 102.32% as shown in **Table 2**. In-vitro in vivo dissolution provides useful information regarding the bioavailability and batch to batch consistency. It is a compendial requirement for the license of the drug. This test is also utilized to specify stability and development characteristics²⁷. It is a surrogate point in development of a formulation and to predict the bioequivalence²⁸. In this study multiple point dissolution were performed to evaluate the maximum drug release. At 30 minutes, the percentage dissolution of the drug was found to be 98.49% to 101.32% of the test formulations as shown in **Figure 1**. Similarity (f_2) and dissimilarity (f_1) factors represent

RESULTS

Table 1: Invitro Quality Parameters Weight variation, Thickness, Diameter, Hardness, Friability, Disintegration and Dissolution of Itopride Hcl (250mg) I1-I5

Codes	weight (mg) Mean ± SD	Thickness (mm) Mean ± SD	Diameter (mm) Mean ± SD	Hardness (kg) Mean ± SD	Friability (%)	Disintegration Time (min) (not >15 min)	Dissolution Studies of Itopride HCl (250mg) in 0.1N HCL at 30 minutes
I1	133±7.32	3.12±0.06	7.3±0.02	8.5±0.17	0.40	5 min 40 sec	99.89
I2	131.5±7.45	2.4±0.04	3.5±0.05	6.12±0.26	0.57	9 min 32 sec	101.32
I3	130.5±6.04	2.05±0.03	7.4 ±0.06	2.59±0.11	0.29	6 min 19 sec	99.87
I4	179.5±14.6	3.1±0.07	8.2±0.02	4.07±0.57	0.37	5 min 22 sec	98.49
I5	311±15.18	3.3±0.02	9.8±0.04	6.6 ±0.62	0.21	2 min 40 sec	99.84

Table 2: Percentage Assay of Several Brands of Itopride Hcl (250mg) available in Karachi, Pakistan Itopride Hcl (250mg) I1-I5

No.of Tablets	I1 (%)	I2 (%)	I3 (%)	I4 (%)	I5 (%)
20	99.98	99.89	101.24	99.67	99.87
20	100.9	102.6	99.96	101.4	103.35
20	99.97	99.90	100.78	100.45	99.99
Mean	100.2	102.32	100.66	100.50	102.24
SD	0.53	0.98	0.64	0.86	0.54

Table 3: Application of model independent approaches similarity factor (f_2) and dissimilarity factor (f_1) on selected brands of itopride 250 mg with I2 brand is selected as Reference (I1-I5)

Similarity (f_2) and Dissimilarity (f_1) factor at 0.1N HCl	I1	I3	I4	I5
f_1	3.16	3.91	6.52	1.86
f_2	76.94	74.55	61.80	84.87

Table 4: Application of kinetic models (model dependent approaches) on the multiple Dissolution data of the selected brands Itopride (250 mg) I1-I5

Code	r^2	$k_1(m)$	r^2	$kH(m-1/2)$	r^2	$kHC(m-1/3)$	r^2	B	A
I1	0.9628	0.081	0.9756	17.450	0.9520	0.022	0.9630	1.019	12.971
I2	0.9599	0.085	0.9734	17.902	0.9652	0.023	0.9639	1.086	14.701
I3	0.9699	0.081	0.9663	17.526	0.9776	0.022	0.9767	1.115	16.762
I4	0.9737	0.099	0.9276	18.630	0.9467	0.026	0.9750	1.047	11.306
I5	0.9753	0.008	0.9850	18.045	0.9701	0.023	0.9768	1.051	12.974

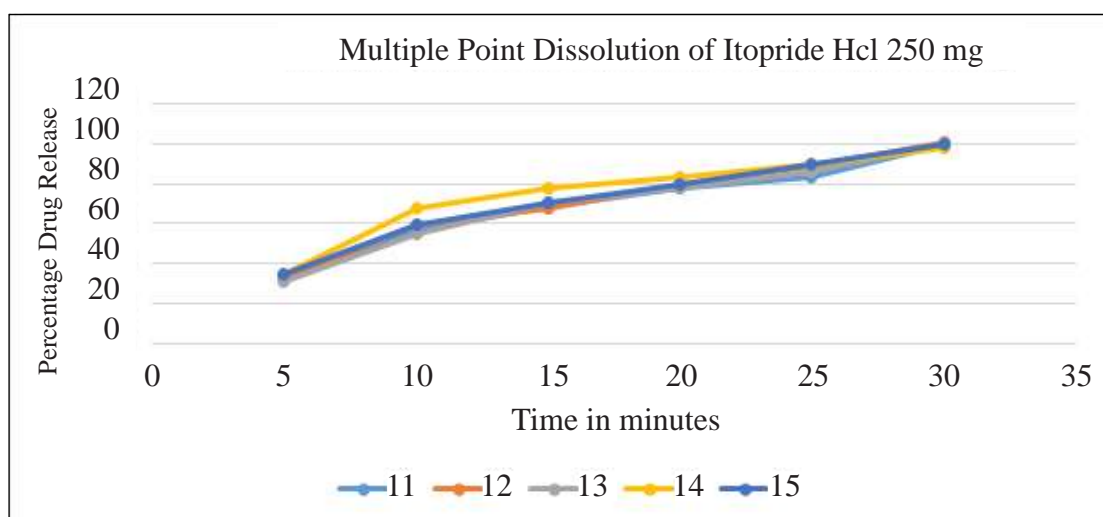


Figure 1: Graphical presentation of multiple point dissolution studies of Itopride HCl 250 mg at 0.1N HCL for 30 minutes

the difference and similarity between the two dissolution profiles²⁹. The difference factor f_1 is also called as Fit factors, and the similarity factor f_2 , first proposed by Moore and Flanner. The factor f_1 quantify the difference in drug released between the dissolution profiles at multiple time points the profiles of the test and reference are identical when f_1 is zero dissimilarity between the two profiles increases, as the value increases and usually a value of 15 or below indicates fair similarity³⁰. The f_2 values are between 0 and 100, and if the calculated value is greater than 50, the test and reference formulations are similar. The similarity factor f_2 is achieving popularity due to its recommendation by regulatory authorities for the assessment of similarity between dissolution profiles³¹. In this study reference formulation was I2 and the test formulations were I1, I3, I4 and I5. (f_2) value was found to be 84.87 to 61.80 and (f_1) value was found to be 1.86 to 3.9 as shown in **Table 3**. In this study several kinetic models (dependent approaches) were applied successfully as shown in **Table 4**.

CONCLUSION

This study demonstrates the importance of rigorous quality control measures in ensuring the efficacy, safety, and reliability of pharmaceutical products. The evaluation of quality control parameters such as weight variation, hardness, thickness, diameter, dissolution, and disintegration, emphasize the importance of following Pharmacopoeial standards. These tests are important in ensuring that each tablet contains the accurate amount of active ingredient, with weight variation and hardness tests authenticates uniformity and mechanical strength, respectively. The rationality of this research lies in the requirements to guarantee that pharmaceutical products meet high quality requirements, thereby optimizing therapeutic outcomes and enhancing patient compliance. This study contributes to the advancement of high-quality pharmaceutical products by highlighting the significance of quality control for both manufacturers and regulatory agencies.

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Knowledge, Attitude, and Practice regarding Geriatric Care among Nurses at Tertiary Care Hospitals in Karachi, Pakistan

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ABSTRACT

Objective: This study aimed to determine the knowledge, attitude, and practice regarding geriatric care among nurses at public and private tertiary care hospitals in Karachi.

Methodology: A descriptive cross-sectional study was conducted at Dr. Ruth K.M Pfau Civil Hospital which is a public setting and at Dr. Ziauddin Hospital in North Nazimabad, which is a private hospital. Registered nurses with a valid license who were working at these two hospitals were the target population. Sample size was calculated by the software 'OpenEpi' version 3.0 using a 95% confidence level with 80% power of the study. The participants were selected using consecutive sampling technique.

Results: Out of all the participants (n=374 nurses), 190 (50.8%) were male and 184 (49.2%) were female. The majority of nurses demonstrated good knowledge 157 (42%), positive attitude 305 (81.6%), and good practice level 289 (77.3%). In addition, most nurses from public hospitals had good geriatric care knowledge 92 (41.4%), positive attitude 181(81.5%) and good practice level 182 (82.0%) compared to a private hospital. This was statistically significant with (p-value<0.05).

Conclusion: Over all, this study found that nurses working in both private and public tertiary care hospitals had good knowledge of geriatric care, a positive attitude, and good practice towards caring for the elderly patients.

Keywords: Attitude, geriatric care, knowledge, nurses, practice

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INTRODUCTION

Currently, the human population is living longer than before, and the majority of people are expected to live for sixty years or more¹. Recently, older population has probably grown due to a reduction in mortality and persistent improvement in survival across the globe². Ageing is a process of growing older that may lead to permanent physical, mental, psychological, and social changes³.

The global population aged sixty years will increase from 1 billion to 1.4 billion in 2030, and it is also estimated that by 2030 one out of six people will be 60 years old or above¹. The increasing number of older people leads to a greater burden on healthcare delivery systems globally⁴. According to the United Nations, in 2017, 39% of hospitalized patients in all healthcare settings were older adults⁵. The ageing process increases the risk of disability, dependence, risk of falls, and death among old people and several conditions such as dementia, osteoarthritis, cataracts, diabetes, depression, hypertension, and other issues⁶. The increase in the elderly's life expectancy means more dependency and greater demand for long-term care due to various health issues associated with ageing⁷. Current demographic and healthcare patterns indicate that the demand for geriatric nurses is growing faster due to the high rate of hospitalization among elderly people⁸. Globally, the nurse shortage with a parallel rise in the proportion of the older population highlights the need to have more nurses specialize in the field of geriatrics

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and be willing to care for older adults⁹. In addition, due to the increasing number of older patients being admitted to hospitals, there is a growing demand for geriatric nurses¹⁰. Nurses have an essential role in recognizing and fulfilling the physical and mental health requirements of elderly people in a range of locations, including their own homes, supported living, nursing home, hospital, or residential care. Nurses with good knowledge, practice and positive attitudes directly affect the quality of care of elderly patients and also play a role in their lifestyle and health¹¹. Hence, this study aimed to determine the level of knowledge, attitude, and practice regarding geriatric care among nurses at private and public tertiary care hospitals in Karachi.

METHODOLOGY

IRB/ERC Approval:

This 7-month study, conducted from February to August 2022, received ethical approval from the Ethical Review Committee of Ziauddin University with reference code: 4680122SKNUR.

A descriptive cross-sectional study design was adopted to determine the knowledge, attitude, and practice regarding geriatric care, of nurses at private and public tertiary care hospitals in Karachi. The study was conducted at two hospitals Dr. Ziauddin Hospital, and Dr. Ruth KM Pfau Civil Hospital, Karachi. Registered nurses working in public and private tertiary care teaching hospitals were the target population. Registered nurses of both genders with valid licenses from the Pakistan Nursing and Midwifery Council, having experience of more than six months, and giving direct care to elderly patients, were included in the study. While, nurses who were not willing to participate, those working part-time, and nurse interns were excluded from the study. The sample size was calculated via online software, OpenEpi version 3.0. For sample size calculation, taking 76.4% proportion of 'good knowledge' among nurses. Considering the aspect of knowledge, the calculated sample size was 277 for this study. In addition, 97 subjects were added for the accuracy of finding and generalization of the results. Thus, the final sample size taken for the study was 374. Subjects were selected through a consecutive sampling method. The research tools 'knowledge, attitude, and practice' were adopted from the previous studies^{12,13}. Regarding the knowledge of the care of the elderly, the tool consists of 28 multiple-choice questions with one right answer. A score of =80% was considered excellent knowledge, a score between 70-80% good knowledge, a score between 60-69% average

knowledge, and a score of <60% was regarded as poor knowledge. The attitude was assessed on a five-point Likert scale, ranging from 'strongly agree' to 'strongly disagree'. All items were scored from 1 to 5, where 5 stands for 'strongly agree' and 1 for 'strongly disagree'. It consisted of 16 items. The practice section involved seven questions to evaluate whether the participants had completed these seven essential nursing care practices for the elderly. Each question in this section had two answers 'did it or not'. The participants could get one score for every practice they completed. The total score ranged from 0 to a maximum of 7.

For the analysis of data Statistical Package for the Social Sciences (SPSS version 24.0) was used. Frequencies and percentages were calculated for all demographic variables. For the inferential statistics, Chi-square test was used to determine associations of knowledge, attitudes, and practices of nurses.

RESULTS

The mean \pm SD age of the participants was found to be 32.1 \pm 5.34 years. Referring to the marital status of the participants, 120 (32.1%) were single, and 231 (61%) were married. Referring to the participants' working hours per day, 123 (32.9%) participants worked for 6 hours, 132 (35.3%) participants worked for 8 hours, and 119 (31.8%) participants worked for more than 8 hours per day (Table-1).

Table 1: Socio-Demographic Characteristics of Study Participants

Variables (n= 374)	Frequency (%)
Gender	
Male	190 (50.8)
Female	184 (49.2)
Marital Status	
Single	120 (32.1)
Married	231 (61)
Widow/widower	6 (1.6)
Divorce	17 (4.5)
Average Working Hours Per Day	
6 hours	123 (32.9)
8 hours	132 (35.3)
More than 8 hours	119 (31.8)
Qualifications of Nurses	
RN Diploma	87 (23.2)
Post RN BSN Degree	145 (38.7)
BSN Degree	142 (37.9)

Table-2 depicts the knowledge, attitude, and practice of nurses regarding Geriatric Care. About the knowledge of nurses regarding geriatric care, the results show that 157 (42.0%) had good knowledge. Out of the total population, 305 (81.6%) participants showed a positive attitude towards geriatric care. Out of the total 374 participants, 289 (77.3%) had good practice of geriatric care.

Table 2: Knowledge, Attitude, and Practice Regarding Geriatric Care among Nurses

Variables	Frequency (%) n=374
Knowledge	
Excellent	56 (15.0)
Good	157 (42.0)
Average	123 (32.9)
Poor	38 (10.2)
Attitude	
Positive	305 (81.6)
Negative	69(18.4)
Practice	
Good	289 (77.3)
Bad	85(22.7)

Table-3 shows the association between the nurses' knowledge of geriatric care in public and private hospitals. Out of the total participants in public hospitals, 56 (25.2%) nurses were found to have excellent knowledge as compared to the nurses in a private hospital, none of whom scored excellent ($p < 0.01$). It was found that more public sector nurses had good knowledge 92 (41.4%) as compared to nurses working in private hospitals 65 (42.8%) with a significance of ($P < 0.01$). Furthermore, it was found that more nurses 65 (42.8%) working in a private hospital had average knowledge as compared to nurses working in a public hospital 58(26.1%) with a significance of ($P < 0.01$). In addition, the majority of nurses working in the private hospital 22 (14.5%) were found to have poor knowledge compared to public hospital nurses 16 (7.2%).

Table 3: Association of Knowledge with Work-setup

Level of Knowledge	Public Hospital Frequency (%) n=222	Private Hospital Frequency (%) n=152	p-value
Excellent	56(25.2)	0(0.0)	<0.01
Good	92(41.4)	65(42.8)	
Average	58(26.1)	65(42.8)	
Poor	16(7.2)	22(14.5)	

The chi-square test was applied for Association. p -value = 0.05 was considered significant

DISCUSSION

Every human goes through the process of ageing. According to most gerontologists, it commences in the fourth decade of life and ends at death. A person goes through diverse changes throughout this period including biological, physiological, and psychosocial changes which lead to the deterioration of the quality of life¹⁴. Therefore, geriatric nursing care is nowadays considered the most popular speciality in the field of nursing, and it needs unique skills and knowledge¹⁵.

Good knowledge, practice, and attitude from nurses in caring for the elderly can improve patient outcomes, as well as satisfaction from patients and families. It can also help caregivers provide older people with the care they need¹⁶. Therefore, the present study intended to assess the knowledge, attitudes, and practices regarding geriatric care among nurses and to compare this knowledge, attitude, and practices regarding geriatric care in nurses of private and public tertiary care hospitals.

The existing literature presents many studies that address nurses' knowledge, attitude, and practice for the care of the elderly in different settings¹⁷. In the current study, a total of 374 nurses were recruited from private and public hospitals. Out of these, 222 (59.3%) worked at a public hospital and the remaining 152 (40.6%) worked at a private hospital. The majority of study participants showed good knowledge 157 (43.6%) related to geriatric care. While among the remaining participants, 123 (32.9%) had average knowledge and 38 (10.2%) had poor knowledge about geriatric care. This shows that the majority of nurses had enough knowledge to care for geriatric patients.

These findings are consistent with a previous study which indicated that an almost similar frequency of knowledge among nurses, with higher frequency lying in average and good levels, and a lower frequency of poor knowledge was found¹⁸. However, contradictory results were also observed in a previous study which depicted that 37.2% nurses had over all good knowledge regarding the care of older people while 62.8% had poor knowledge¹⁷. Similar findings like a higher frequency of poor knowledge were also observed in another study. This difference in results may be the consequence of educational backgrounds and academic qualifications. The majority of the participants in the present study were Post RN 145 (38.7%) and BSN 142 (37.9%), and fewer were diploma holders 87 (23.2%). While, in the studies mentioned above, the participants had poor educational backgrounds and diploma level of education¹⁹.

The current study shows positive attitudes among nurses towards the care of older patients were more frequent (81.6%) as compared to negative attitudes (19.1%) These results show the encouraging and favourable attitude of the majority of nurses. Additionally, it also showed that good knowledge may have a significant role in the positive attitude toward geriatric care. Similarly, according to the findings of a previous study conducted in Pakistan, 60% of nurses had an average knowledge score and (51.8%) a favourable positive attitude score towards care for older individuals, indicating that better knowledge may lead to a favourable attitude¹⁸. The finding of the recent study was contradictory to a previous study in which the nurses' attitude was unfavourable towards elderly care due to a lack of knowledge¹⁷.

The current study indicates that the majority of nurses (77.3%) have a good level of practice towards geriatric care. On the other hand, a study found that 84% of participants do not practice good geriatric care²⁰. It shows a higher frequency of bad practices as compared to knowledge and attitude, which could be due to the lack of training regarding geriatric care.

CONCLUSION

Overall, this study concluded that the nurses from the public sector demonstrated good knowledge more frequently as compared to the nurses of private hospitals, but the attitude and practice were observed to be slightly better in private hospital nurses.

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Conflict of interest: Authors declare that there is no conflict of interest.

Authors' Contributions: **SK:** Conceptualization of the study, data collection, and analysis **SK:** Supervision, review of the manuscript, conducting literature reviews, and overseeing data collection **R:** Drafting the manuscript and participating in the editing process

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A Survey of Perioperative Monitoring of Body Temperature and Various Techniques Used in Warming the Patients

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ABSTRACT

Objective: To determine the frequency of patient warming and the various techniques used for patient warming in different tertiary care hospitals of Karachi Pakistan

Methodology: The present study was a one-year cross-sectional study conducted at three different hospitals in Karachi in which 80 anaesthesiologists (consultants, specialists and final-year residents) were included through non-probability sampling. The data on perioperative temperature recording and warming methods, including blankets, forced-air warming, and infusion of warmed fluids, were retrieved through a self-administered questionnaire. This study was conducted to evaluate the knowledge and practices of anaesthesiologists in preventing intra- or perioperative hypothermia and its consequences, and informed verbal consent was obtained from all participants. The SPSS version 26 was used to analyze the data. Descriptive statistics were calculated, and the data was analyzed and generated with 95% confidence interval by using SPSS version 26.

Results: The mean age of the participants was found to be 40.13 ± 13.09 years, among them 63.7% were male. The practice of patient warming was documented in 70% of participants. Ambient temperature adjustment was noted as the most common technique, used by 83.3% of the warming group as compared to 16.7% of the non-warming group ($p=0.121$). Forced-air warming (59.1% vs. 40.9%, $p=0.832$) while intravenous fluid warming was documented in (62.5% vs. 37.5%, $P > 0.05$). Temperature monitoring was inconsistent, with over 50% of patients never monitored during surgery or recovery.

Conclusion: The results show that there is variability in perioperative temperature monitoring and warming practices among anaesthesiologists in Karachi. While use of patient warming was widespread, application methods varied considerably. Ambient temperature adjustment was an overused technique; however, practices such as forced-air warming and fluid warming were mixed. These findings highlight the need for standardization of protocols and increased clinician awareness in order to prevent perioperative hypothermia and its associated complications.

Keywords: Clinical protocols, patient warming, surgical procedures, warming techniques

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INTRODUCTION

Perioperative monitoring of body temperature is an essential element of surgical care, as it directly influences the results of patients. Maintaining normothermia during surgical procedures considerably reduces the risk of complications such as surgical infections (SSIS), cardiovascular events, and prolonged recovery times¹. Hypothermia, defined as a central

body temperature less than 36°C, is often induced inadvertently during surgery due to factors such as exposure to cold operating rooms, administration of anaesthetics, and the use of cold intravenous liquids². The importance of body temperature monitoring, lies in its role in the detection of these temperature drops, which can lead to unfavorable results if not treated quickly³.

Various techniques are used to maintain the temperature and rewarm the patients' bodies during surgical procedures. These methods can be classified as active and passive warming strategies. The methods of active warming, such as warming covers of forced air, proved to be effective in preventing hypothermia during surgery⁴. A systematic journal indicated that the use of these devices not only maintains the central

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temperature but also improves postoperative analgesic and clinical results⁵. Another study highlighted the significance of warming patients before surgery, demonstrating that preoperative warming markedly minimizes intraoperative temperature drops⁶.

Conversely, passive methods for warming, like hot socks or blankets, also contribute to temperature maintenance, but their efficiency is variable⁷. Passive warming can have benefits, however it is typically unable to provide the same amount of thermal control as active warming techniques⁸. Therefore, many institutions are now using hybrid strategies that combine both active and passive techniques to optimize thermal comfort and pulsatility around the perioperative period.

Specifically, global recommendations emphasize the use of full thermal control in operating rooms⁹. During the surgical procedure, body temperature needs to be continuously monitored and real-time feedback systems allow prompt corrective actions to overcome hypothermia¹⁰. The performance of the anaesthesiologist on this question underlines the need for alertness concerning temperature management, because their interventions directly influence patient safety¹¹.

Effective monitoring and management of perioperative body temperature are essential to minimize complications related to hypothermia. The use of a combination of active, and passive warming techniques, as well as in-depth monitoring practice is necessary to optimize the patient results during the surgeries¹²⁻¹⁴. The importance of this aspect of surgical care cannot be underestimated, as it contributes to the improvement of recovery trajectories and the overall satisfaction of patients.

METHODOLOGY

IRB/ERC Approval:

This cross-sectional study was conducted over six months at various tertiary care hospitals in Karachi, after obtaining approval from the Institutional Review Board of Indus Hospital & Health Network (Ref No: IHHN-IRB-2021-09-002).

A cross-sectional study was conducted over a period of six months at various tertiary care hospitals in Karachi, including The Indus Hospital, Aga Khan University Hospital, Civil Hospital, Jinnah Postgraduate Medical Centre, Liaquat National Hospital, Sindh Institute of Urology and Transplant, Patel Hospital, and Abbasi Shaheed Hospital.

The sample was composed of 80 anaesthesiologists, including consultants, specialists, and the final-year

residents in anaesthesiology. The participants were selected using a non-probability consecutive sampling technique. Inclusion criteria for the study were anaesthesia consultants, specialists, and final-year anaesthesia residents. Participation was voluntary. The exclusion criteria included anaesthesia residents in their first, second, or third years of training, MCPS residents, and anaesthesiologists working in primary and secondary care hospitals.

"Practicing anaesthesiologists currently working in tertiary care hospitals were further classified into three categories: consultants, specialists, and final-year residents in anaesthesiology. Perioperative body temperature monitoring refers to the process of measuring a patient's body temperature during the perioperative period (preoperative, intraoperative, and postoperative phases) to prevent complications associated with hypothermia. This includes using thermocouple probes at the tympanic membrane, axilla, rectum, and forehead skin surface, and measuring tympanic temperature using infrared devices, and forehead temperature using liquid crystal thermometer strips. Techniques of patient warming include various methods to prevent or treat hypothermia during the perioperative period. These techniques include the use of simple cotton blankets, carbon-fiber sheets, circulating hot water mattresses, forced air warming, warm fluid infusion, and increasing the ambient temperature in the operating room or recovery area.

Verbal Informed Consent was obtained and those who agreed to take part in the study were provided with a printed self-administered questionnaire which was designed to gather information on demographic details such as the participant's age, qualifications, and the type and part of the hospital where they were working. In addition, the survey included questions regarding the methods used by the anaesthesiologists to assess and monitor body temperature. Beyond monitoring temperature, details were sought on warming methods used during the perioperative periods.

The questionnaire formulated for the survey was analyzed to assess the anaesthesiologists' practical and knowledge-based approaches to the prevention and management of perioperative hypothermia and its associated complications.

Descriptive statistics were calculated, and data was analyzed on SPSS version 26 and generated with 95% confidence interval. Mean with standard deviation, while frequency with percentage, were calculated for quantitative and qualitative variables respectively. The Chi-square test was applied to compare the warming and non-warming techniques at 5% level of significance.

RESULTS

Demographic characteristics of respondents indicated a mean age of 40.13 ± 13.09 with 60% aged between 27 and 40 years and the rest were older. As many as 63.7% of the respondents were male and 36.3% were female. The mean years of professional experience was 7.33 ± 4.32 (97.5% = 1–15 years of professional experience; 2.5% > 15 years). In terms of qualifications, 52.5% were consultants with FCPS, 7.5% MCPS specialists, 1.3% had American Board certification, while 2.5% were FCPS residents with < 2 years of experience and 36.3% with = 2 years of experience. Of these, 76.3% were from private hospitals, while 23.8% were affiliated with government hospitals. Professionals worked mainly in operating rooms (98.8%), and only 1.3% in intensive care unit. This data highlights a predominance of middle-aged, male professionals with less than 15 years of experience, primarily working in operating rooms within private hospitals (Table I).

Table 1: Demographic Characteristics of the Respondents

Variable	Frequency (%)
Age, Mean \pm SD= 40.13 \pm 13.09 Years	
27 - 40 Years	48 (60.0)
>40 Years	32 (40.0)
Gender	
Male	51 (63.7)
Female	29 (36.3)
Years of Experience, Mean \pm SD= 7.33 \pm 4.32 Years	
01 - 15 Years	78 (97.5)
>15 Years	2 (2.50)
Qualification	
FCPS (Consultants)	42 (52.5)
MCPS (Specialist)	6 (7.5)
American Board (Certified Diploma)	1 (1.3)
FCPS resident < 2 years' Experience	2 (2.5)
FCPS resident = 2-year experience	29 (36.3)
Type of Institute	
Private Hospital	61 (76.3)
Government Hospital	19 (23.8)
Place of Working at Hospital	
Operating Room	79 (98.8)
Intensive Care Unit	1 (1.3)

Comparison of different warming methods and their relation to warming and non-warming patient groups was studied. Out of all the techniques, increasing the

ambient temperature was most commonly employed (83.3% vs 16.7% in the warming vs non-warming groups respectively; OR: 3.0, CI: 0.786–11.44, $p=0.121$). Hot water bags were not used in the warming group but one was employed in the non-warming group (p -value not applicable, OR: 2.3, CI: 2.30–2.03). Regarding electrical blankets, their use was noted for 60% in the warming group and 40% of non-warming group (OR:0.9, CI:0.115–7.03, $p=0.920$). The forced-air warming systems was used by 59.1% in the warming group, and only 40.9% in the non-warming group (OR: 0.867, CI: 0.231–3.25, $p=0.832$). Finally, warming of IV fluids was recorded in 62.5% of the warming and 37.5% of the non-warming group (Table 2).

Patient warming and temperature monitoring practices show wide gaps and differences. Only 5% of patients in the operating room at 15 minutes and 12.5% at 30 minutes had their temperatures assessed, and 52.5% were never recorded. Temperature monitoring in the recovery unit: 3.8% at 15 minutes, 7.5% at 30 minutes, and 58.8% never monitored. Axilla was the most frequent site of temperature measurement (46.3%), while tympanic membrane (21.3%) and rectum (2.5%) were measured less frequently. Perioperative warming was reported as practiced 'sometimes' in 67.5% of cases, and patients were reported to have not been warmed at all in 32.5% of cases. Pulmonary artery (36.3%) and tympanic membrane (28.7%) were regarded as the most accurate core temperature measurement sites followed by skin (5%). Total 21.3% percent had a body temperature of 37.5°C during transport from recovery unit to ward (and 48.8%, 36°C). These findings emphasize a need for common reference established protocols for temperature monitoring and warming procedures (Table 3).

Differences in patient-associated parameters among healthcare professionals using different methods of warming were based on age, gender, years of experience, qualifications, workplace, and type of institute. Increasing ambient temperature was the most common method used among patients aged 27–40 years (56.3%), followed by forced-air warming systems (18.8%) and warming of intravenous fluids (16.7%). In professionals older than 40 years, forced-air warming (40.6%) and warming of IV fluids (25.0%) were more popular. More men used forced-air warming systems (31.4%) and warming of intravenous fluids (23.5%), while more women preferred to increase the ambient temperature (55.2%).

Increasing the ambient temperature (46.2%) was the most common use among professionals with 1–15 years of experience, whereas 100% in the group of professionals with more than 15 years of experience,

Table 2: Comparison between Patients Warming and the Techniques Used

Techniques	Patients Warming		OR (C.I.)	P-Value
	Warming (n=56)	Non-Warming (n=24)		
Increase the ambient temperature	30 (83.3)	6 (16.7)	3.0 (0.786—11.44)	0.121
Hot water bags	0 (0.0)	1 (100.0)	2.3 (2.30—2.03)	—
Electrical blanket	3 (60.0)	2 (40.0)	0.9 (0.115—7.03)	0.920
Forced-air warming systems	13 (59.1)	9 (40.9)	0.867 (0.231—3.25)	0.832
Warming of the intravenous fluids	10 (62.5)	6 (37.5)	—	—

Applied Chi-Square, Fisher’s Exact test & Regression Model, OR (Odd Ratio), C.I. (Confidence Interval)

Table 3: Practices in Patient Warming

	At 15 mins	At 30 mins	Never
Body temperature of the patient in the operating room	4 (5.0)	10 (12.5)	42 (52.5)
Body temperature of the patient in the recovery unit	3 (3.8)	6 (7.5)	47 (58.8)
	Tympanic Membrane	Rectum	Axilla
Measure the body temperature	17(21.3)	2(2.5)	37(46.3)
	Yes, sometimes	No	-----
Warm up the patients in the perioperative period	54(67.5)	26(32.5)	0(0.0)
	Tympanic Membrane	Skin	Pulmonary Artery
Site showing the most accurate measurement of core temperature	23(28.7)	4(5.0)	29(36.3)
	37.5°C	36°C	-----
Body temperature of the patient for transporting the recovery unit to the ward bed	17(21.3)	39(48.8)	0(0.0)

Table 4: Distribution of Patients Related Parameters Among Healthcare Professionals’ Preferred Techniques

Parameters		Increase the ambient temperature	Hot water bags	Electrical blanket	Forced-air warming systems	Warming of the intravenous fluids
Age (years)	27 – 40	27(56.3)	1(2.1)	3(6.3)	9(18.8)	8(16.7)
	>40	9(28.1)	0(0.0)	2(6.3)	13(40.6)	8(25)
Gender	Male	20(39.2)	1(2.0)	2(3.9)	16(31.4)	12(23.5)
	Female	16(55.2)	0(0.0)	3(10.3)	6(20.7)	4(13.8)
Experience (years)	1 – 15	36(46.2)	1(1.3)	5(6.4)	20(25.6)	16(20.5)
	>15	0	0	0	2(100)	0
Qualification	FCPS (Consultants)	19(45.2)	1(2.4)	1(2.4)	13(31)	8(19)
	MCPS (Specialist)	3(50)	0	1(16.7)	1(16.7)	1(16.7)
	American Board (Certified Diploma)	1(100)	0	0	0	0
	FCPS resident < 2 years’ Experience	0	0	0	1(50)	1(50)
	FCPS resident = 2-year experience	13(44.8)	0	3(10.3)	7(24.1)	6(20.7)
Type of Institute	Private	28(45.9)	1(1.6)	5(8.2)	16(26.2)	11(18.0)
	Government	8(42.1)	0	0	6(31.6)	5(26.3)
Place of Working	Operating Room	36(45.6)	1(1.3)	5(6.3)	22(27.8)	15(19)
	ICU	0	0	0	0	1(100)

ICU (Intensive Care Unit), FCPS (Fellow of College of Physicians and Surgeons), MCPS (Member of College of Physicians and Surgeons)

used forced-air warming systems. FCPS consultants most frequently reported raising ambient temperature (45.2%) and using forced-air warming systems (31.0%), while FCPS residents with =2 years of experience reported increasing ambient temperature (44.8%) and using forced-air warming systems (24.1%). Increasing the ambient temperature (45.9%) and forced-air warming systems (26.2%) were common in private hospitals, whereas in government hospitals, forced-air warming systems (31.6%) and intravenous fluids warming (26.3%) were more frequent.

Increasing the ambient temperature (45.6%) and forced-air warming systems (27.8%) were the most commonly used by professionals in the operating room. In contrast, the only method used in the ICU was warming of intravenous fluids (100%). These results demonstrate the diversity of practice driven by patient characteristics, professional background, and work environment, and highlight a lack of uniformity in warming protocols (Table 4).

DISCUSSION

Perioperative temperature regulation and management, as well as warming methods applied to patients, are very important to reduce the risk of intraoperative hypothermia, a widely observed but avoidable dangerous state which may provoke many other complications. The aim of this discussion was to assess how often patient warming practices are performed, the methods used and the impact these perioperative measures have on patient outcomes.

Hypothermia during surgery has been long-documented, as there are multiple patients whose temperature drops as a result of the administration of anaesthesia and cooling effects as a consequence of the environment of the operating room. Perioperative hypothermia is associated with significant risk. Hypothermia can be associated with prolonged recovery times, higher blood loss, increased infection rates, and increased post-operative care¹⁵. In addition, it causes serious cardiovascular and metabolic disturbance which may hinder surgical procedure. In the study by Yi et al., despite this intravesical therapy, the authors stress the need for active temperature management because they also highlight the high prevalence of intraoperative hypothermia, with a large proportion of general anaesthetized patients experiencing a decrease in the core body temperature¹⁶.

This has led to advances in techniques to prevent hypothermia during surgery. Perioperative active warming techniques (such as forced-air warming blankets) are in widespread use to prevent perioperative

hypothermia and maintain thermal comfort. In their study, Özsaban and Acarođlu showed that active warming (forced-air warming blankets) provides significant reduction of postoperative hypothermia and enhanced thermal comfort¹⁷. Not only are these methods effective in preventing hypothermia but they also result in improving surgical outcomes, quicker recovery, and reduction in postoperative morbidity.

There are a variety of warming methods, which depend on the type of surgery, patient characteristics, and institutional protocols. For example, specifically in the field of colorectal surgeries, Gala et al. call for more occasional usage of warming devices to actually avoid hypothermia, especially in high-risk patients (old patients, long procedures)¹⁸. The study also found that passive warming techniques, such as warm intravenous fluids or warm air circulation, are sometimes insufficient for high-risk surgeries, necessitating the use of more robust interventions like forced-air warming or circulating water garments.

Furthermore, the warming technique's efficacy can be influenced by the temperature at which the device is set. In an experimental study based on different temperatures of forced-air warming blanket, He et al found that higher ambient temperatures are better than lower temperatures for reducing inflammatory markers and improving postoperative clinical recovery in paediatric patients undergoing congenital hip dislocation procedures¹⁹. This suggests that tailoring the warming method to the patient's condition and the specifics of the surgery is essential for optimizing outcomes.

Despite the widespread use of warming techniques, the implementation of patient warming practices is often inconsistent. According to a survey by Inal et al. among anesthesiology specialists in Turkey, although the majority of clinicians were aware of the risks of hypothermia, prevention practices varied significantly²⁰. Notably, barriers to successful and sustained implementation of warming included less education on warming, insufficient apparatus, and heterogeneity of protocols between institutions. These findings align with those of Munday et al., who identified several barriers and enable the implementation of perioperative hypothermia prevention, with the multidisciplinary team's involvement and training being central to overcoming these barriers²¹.

The strength of the current body of literature is its demonstration of the effectiveness of active warming techniques in preventing hypothermia and improving patient comfort. The most reliable evidence of the clinical benefits of these practices, such as reduced postoperative complications and shorter recovery time,

comes from their randomized controlled trials, for example, those of Özsaban and Acarođlu¹⁷. However, one drawback is that many of the studies are based on certain patients' groups or the type of surgery, so the results are not always generalizable. The evidence on the best temperatures and the duration for warming is also inconsistent, which complicates the creation of standardized protocols.

Recommendations for improving perioperative warming practices include standardizing the use of warming techniques across all types of surgery and patient demographics. More consistent training for healthcare providers and access to proper warming devices is needed from institutions. In addition, the ideal warming temperatures and times for individual surgical procedures should be identified and the impact of normothermia during surgery on long-term results should be addressed in future investigations. Patient-specific factors, including age, comorbidities, and surgery duration, can be included in the warming strategy to make it more tailored and potentially more effective.

In our study, the frequency of patient warming was 70% and the techniques used for patient warming were as follows: increase the ambient temperature 83.6% ($p=0.121$), hot water bags 0%, electrical blanket 60% ($p=0.920$), forced-air warming systems 59.1% ($p=0.832$), and warming of the intravenous fluids 62.5%. Inal MA, et al found the warming techniques to be hot air blowing systems and cotton materials such as blankets and socks (63.7% and 20.1%), respectively²⁰. In another study, patient warming was observed in 62.5%, and the techniques were: forced-air warming devices (42.3%), ambient temperature (21.8%), warming the intravenous fluids (13.1%), electrical blankets (12.5%), and hot water bags (8.1%)²².

Perioperative hypothermia or inadvertent hypothermia represents a significant problem with multiple comorbidities ranging from increased morbidity, mortality, prolonged hospital stays, and higher health resource use costs, thus, patient warming is vital in preoperative, intraoperative, and postoperative settings. There is strong evidence for active warming methods, but further work is necessary to address barriers to implementation and optimize approaches for different surgical settings. Ensuring that this is exercised universally and tailored to the individual patient's needs, can tremendously promote patient safety and comfort during surgical procedures.

CONCLUSION

The results show that there is variability in perioperative temperature monitoring and warming practices among anaesthesiologists in Karachi. While use of patient warming was widespread, application methods varied considerably. Ambient temperature adjustment was an overused technique; however, practices such as forced-air warming and fluid warming were mixed. These findings highlight the need for standardization of protocols and increased clinician awareness in order to prevent perioperative hypothermia and its associated complications.

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Harnessing the Healing Power of Java Plum (*Syzygium Cumini*) in an Animal Model: A Study on the Effects Against Neuroinflammation

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ABSTRACT

Objective: This study aims to evaluate and compare the anti-inflammatory, antidepressant, and anxiolytic effects of *Syzygium cumini* (Java plum) pulp and leaf extracts in rodent models, providing evidence-based insights into their neurotherapeutic potential.

Methodology: Adult rodents models (mice and rats) were used in established behavioral and inflammatory models. The Forced Swim Test (FST) assessed antidepressant activity, the Elevated Plus Maze (EPM) tested anxiolytic effects, and the Paw Edema test evaluated anti-inflammatory properties. *Syzygium cumini* pulp and leaf extracts both were administered for comparison of their effects.

Results: Both pulp and leaf extracts showed significant anti-inflammatory effects in the Paw Edema test. The pulp extract reduced immobility time in the FST, indicating antidepressant activity, and exhibited anxiolytic effects in the EPM. The leaf extract only showed anti-inflammatory effects.

Conclusion: This research provides substantial evidence that the pulp extract of *Syzygium cumini* has multifunctional neurotherapeutic potential and is effective in managing neuroinflammation and related mood disorders in animal models. The leaf extract, on the contrary, possesses only anti-inflammatory properties. These results demonstrate that pulp extract is a natural option for creating advanced treatments for mood and neuroinflammatory disorders. Further pharmacological and clinical studies are needed to explore its standardized dosage, as well as assess the long-term effectiveness.

Keywords: Anxiolytic, anti-inflammatory, neuro-inflammation, *syzygium cumini*

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INTRODUCTION

"To eat is a necessity, but to eat intelligently is an art" -La Rochefoucauld¹.

In recent years, the field of nutrition is paying more attention to health-protective functional foods because they promote health and reduce the expense and dependence on synthetic drugs. Since ancient times,

people have utilized plants to treat and cure a wide range of medical issues².

A diet rich in fruits and vegetables enhances overall health and well-being, including mental health. Different patterns of fruit intake exhibit various physiological effects, such as anti-inflammatory and antidepressant-like activities³. Fruits and vegetables are excellent sources of essential nutrients, including minerals (calcium, iron, magnesium, and potassium), dietary fiber, phytochemicals (such as polyphenols), and vitamins (folate, B6, C, and E)

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Oxidative stress can be minimised by vitamins E, C, and polyphenols, that has oxidation inhibitor characteristics.

Majority nutrients within fruits and vegetables may lower inflammation, which lowers the risk of depression⁴

Java plum

Figure 1: Java Plum (Java Plum Fruits and Leaves)



"*Syzygium cumini* (L.), also referred to as black plum, Java plum, Indian blackberry, jamun, jambul, and jambolao, is a species from the Myrtaceae family. Its alternative botanical names include *Eugenia jambolana*, *Syzygium jambolana*, and *Eugenia cuminii*"⁵.

The Java plum is a popular fruit that grows all across the world. Despite its small size, it is rich in organic antibacterial and antioxidant substances⁶.

Syzygium plants are traditionally used to manage various ailments, particularly diabetes. The leaves of some species, as well as the roots, fruit, seeds, and bark of others, are used medicinally⁷.

Jamun fruits are ideal supply of minerals, calcium, phosphorus, iron, protein, also carbs. The jamun fruit is primarily used for its significant mineral content, high anthocyanin concentration, and vitamin C content⁸.

Java Plum leaves are a good source of vitamin A, magnesium, phosphorus, zinc, iron, calcium, chromium, sodium, minerals and easily digested carbs. Additionally, the leaves are utilised in Ayurvedic remedies⁹.

"Its leaves are rich in various bioactive compounds, including flavonoids, alkaloids, phenolics, terpenes, glycosides and tannins. They also comprise of flavonol glycosides like myricetin, quercetin, myricitrin and kaempferol, along with phenolic acids like ferulic acid, gallic acid, and ellagic acid"¹⁰.

However, there aren't enough comparative in vivo research studies comparing pulp and leaf extracts, especially when it comes to how they affect neuroinflammation, depression, and anxiety. Thus, using approved rodent behavioral models, the current work attempts to investigate and contrast their anxiolytic, antidepressant, and anti-inflammatory properties. It is anticipated that this direct comparison

would help clarify which portion of the plant has the best therapeutic potential, and provide fresh perspectives on plant-based neurotherapeutic approaches.

METHODOLOGY

IRB/ERC Approval:

The study was approved by the Institutional Bioethical Committee (IBC) of the University of Karachi under approval number **IBC KU-485/2024**. All procedures complied with the National Research Council's guidelines for laboratory animal care and use, ensuring minimal distress.

Methanol, carrageenan, imipramine, alprazolam, and acetylsalicylic acid were the analytical-grade chemicals employed in this investigation; they were all purchased from regionally approved sources in Pakistan. The digital plethysmometer, rotary evaporator, Soxhlet extractor, and behavioral test apparatus (FST and EPM) were all calibrated before use and kept in standard laboratory conditions.

For our study, we purchased mice weighing between 20 and 25 grams and rats ranging from 150 to 200 grams from THE HEJ Research Institute of Chemistry. The mice were kept in four groups and rats in three groups at a temperature of 28-30°. Mice and rats were fed standard food with free access to tap water.

Experimental Drug Group Protocol for Depression, Anxiety, and Inflammation

Control Group: Normal Saline at a dose of 0.9%.

Standard Group: Treated with 25 mg/kg Imipramine, 0.5 mg/kg Alprazolam, and 300 mg/kg Acetyl Salicylic Acid.

Test Group 1: Java plum pulp extract administered at a dosage of 200 mg per kg of body weight.

Test Group 2: Java plum leaves extract administered at a dosage of 300 mg per kg body weight.

Forced Swim Test

The forced swim test (FST), for examining depressive-like behavior in rodents, is based on the idea that an animal in water would initially try to escape but then show signs of immobility that could be assessed as behavioral despair¹¹. The test result was thought to be the duration of time spent motionless aside from the movements required to keep the nose above water, since this is reduced by a variety of antidepressants¹².

Elevated Plus Maze Test

The Elevated Plus Maze (EPM) evaluates animals' anxiety-like behavior. It has two arms that are open and two that are closed, joined by a central platform. The walls of closed arms are 17 cm high, but those of open arms are exposed. Time spent in each arm served as a measurement of anxiety, and avoiding open arms was a sign of fear¹³.

Paw Edema Test

The method of Winter et al.¹⁴ was used to induce paw edema with carrageenan¹⁵. Paw edema was induced using carrageenan, as mentioned by Winter et al. Researchers commonly employ the consistent technique of paw edema brought on by intraplantar injection of the seaweed polysaccharide carrageenan to assess the anti-inflammatory effects of medications in cases of acute inflammation¹⁶.

Syzygium cumini leaves were collected, dried for 14 days at 40°C, and powdered. Methanolic extraction of 400 g powder was performed using a Soxhlet apparatus, concentrated at 65°C, and dried to a constant weight. The yield was 8.98% w/w and stored under refrigeration¹⁷.

Male mice (30–32g) were procured from the HEJ Research Institute of Chemistry and kept in four groups, while rats were in three groups. They were accommodated in a 12-hour light and 12-hour dark cycle, with the temperature stabilized between 26°C and 28°C.

The data were examined using SPSS software, applying a one-way ANOVA followed by a post hoc Tukey test. A significance level of $P < 0.05$ was classified statistically significant, while $P < 0.01$ indicated high significance, and $P > 0.05$ was regarded as non-significant.

RESULTS

Forced Swim Test: Statistical Interpretation

The Forced Swim Test showed significant differences in immobility times between the Standard and Control, and Standard and Test 2 (leaves extract) groups. Test 1 (pulp extract) exhibited no significant difference compared to the Standard group, suggesting comparable antidepressant effects. One-way ANOVA and Tukey's test confirmed that the pulp extract significantly reduced immobility time, supporting its antidepressant potential.

Elevated Plus Maze Test: Statistical Interpretation

The Elevated Plus Maze Test demonstrated notable differences in the duration spent in the open arms

between the Standard and Control, and Standard and Test 2 (leaves extract) groups. No difference was found between the Standard and Test 1 (pulp extract), indicating anxiolytic effects of the pulp extract. One-way ANOVA and Tukey's test confirmed that the pulp extract notably prolonged the time spent in the open arms, indicating its anxiolytic effects.

Paw Edema: Statistical Interpretation

The anti-inflammatory effects of the Standard drug were compared with the Control, Test 1 (pulp extract), and Test 2 (leaves extract) groups. Notable differences were observed between the Standard and Control groups, but no differences were found between the Standard and Test 1 or Test 2 groups. Both extracts demonstrated anti-inflammatory effects similar to the Standard drug. One-way ANOVA showed significant differences, and post hoc Tukey test confirmed that the Standard group had a greater reduction in inflammation compared to the Control, while the pulp and leaves extracts had comparable effects to the Standard.

DISCUSSION

The Java plum plant is known to contain a variety of phytochemicals, the majority of which have been shown to have beneficial effects on health⁶.

Various physiologically active compounds can be found in a plant's leaves, seeds, peel, and pulp, among other parts¹⁸.

S. cumini is a nutritionally and medicinally significant fruit, rich in calories, lipids, proteins, fiber, carbohydrates, minerals, and vitamins. Its fruits are high in calories, lipids, proteins, fiber, carbohydrates, and mineral and vitamin content. Research indicates that because of its high antioxidant activity, it may have health benefits¹⁹.

This study examined Java plum's (*Syzygium cumini*) neuroprotective, anti-inflammatory, anxiolytic, and antidepressant effects in rodents. The pulp and leaf extracts showed strong anti-inflammatory properties, with the pulp also demonstrating notable anxiolytic and antidepressant effects. These benefits are likely due to Java Plum's high levels of bioactive compounds, such as flavonoids, tannins, and anthocyanins, known for their neuroprotective and antioxidant properties²⁰.

These findings are consistent with previous studies that demonstrated that specific substances, including quercetin, which is found in *Syzygium cumini*, can inhibit oxidative stress and neuroinflammatory pathways, improving behavioral outcomes in animal models.

Table 1: Comparison of Antidepressant effect on Immobility Time by Forced Swim Test among Control group, Standard, Test 1 and Test 2 by using One Way ANOVA and Multiple Comparison post hoc Tukey Test

ANOVA						
immobility time						
	Sum of Squares	Df	Mean Square	F	Sig.	
Between Groups	344.429	3	114.810	134.375	.000	
Within Groups	44.429	52	.854			
Total	388.857	55				
Multiple Comparisons						
Dependent Variable: immobility time						
Tukey HSD						
(I) treatment of drugs	(J) treatment of drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Standard	6.143*	.349	.000	5.22	7.07
	test 1 (pulp)	5.786*	.349	.000	4.86	6.71
	test 2 (leaves extract)	2.929*	.349	.000	2.00	3.86
Standard	Control	-6.143*	.349	.000	-7.07	-5.22
	test 1 (pulp)	-.357	.349	.737	-1.28	.57
	test 2 (leaves extract)	-3.214*	.349	.000	-4.14	-2.29
test 1 (pulp)	Control	-5.786*	.349	.000	-6.71	-4.86
	Standard	.357	.349	.737	-.57	1.28
	test 2 (leaves extract)	-2.857*	.349	.000	-3.78	-1.93
test 2 (leaves extract)	Control	-2.929*	.349	.000	-3.86	-2.00
	Standard	3.214*	.349	.000	2.29	4.14
	test 1 (pulp)	2.857*	.349	.000	1.93	3.78

*. The mean difference is statistically significant at the 0.05 level

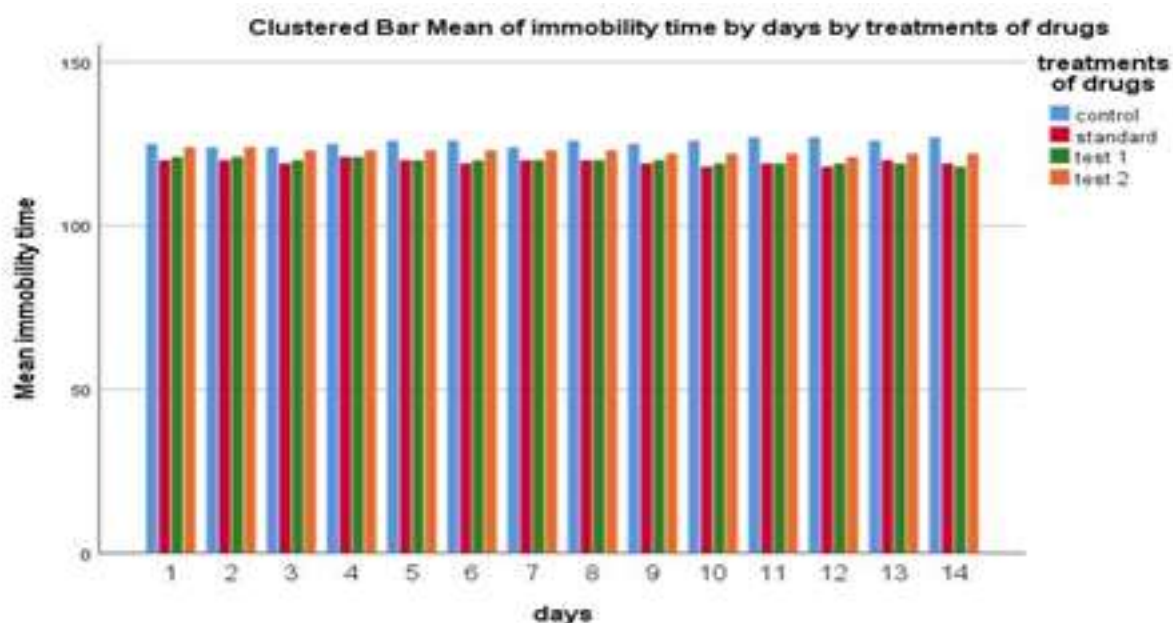


Figure 2: Illustrates the reduction in immobility times, with the pulp extract and Standard group showing significant decreases compared to the Control and leaves extract groups.

Table 2: Comparison of Anxiolytic effect by using Elevated Plus Maze Test among Control group, Standard, Test 1 and Test 2 by using One way ANOVA and Multiple Comparison post hoc Tukey Test

ANOVA						
time spent in open arms						
	Sum of Squares	Df	Mean Square	F	Sig.	
Between Groups	132022.482	3	44007.494	95.400	.000	
Within Groups	23987.357	52	461.295			
Total	156009.839	55				
Multiple Comparisons						
Dependent Variable: time spent in open arms						
Tukey HSD						
(I) treatment of drugs	(J) treatment of drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Standard	-100.000*	8.118	.000	-121.55	-78.45
	test 1 (pulp)	-72.286*	8.118	.000	-93.83	-50.74
	test 2 (leaves extract)	16.500	8.118	.189	-5.05	38.05
Standard	Control	100.000*	8.118	.000	78.45	121.55
	test 1 (pulp)	27.714*	8.118	.007	6.17	49.26
	test 2 (leaves extract)	116.500*	8.118	.000	94.95	138.05
test 1 (pulp)	Control	72.286*	8.118	.000	50.74	93.83
	Standard	-27.714*	8.118	.007	-49.26	-6.17
	test 2 (leaves extract)	88.786*	8.118	.000	67.24	110.33
test 2 (leaves extract)	Control	-16.500	8.118	.189	-38.05	5.05
	Standard	-116.500*	8.118	.000	-138.05	-94.95
	test 1 (pulp)	-88.786*	8.118	.000	-110.33	-67.24

*. The mean difference is statistically significant at the 0.05 level

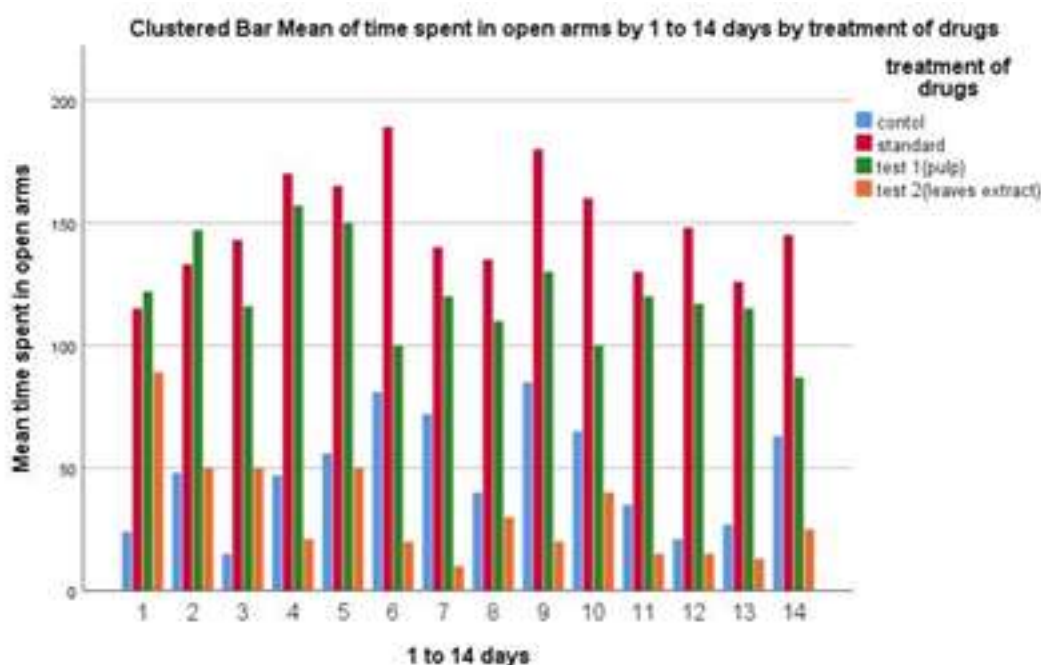


Figure 3: Illustrates the EPM results, emphasizing the increased time duration in the open arms by the pulp and Standard group compared to other groups.

Table 3: Anti-inflammatory effect by using Paw Edema Test among Control group, Standard, Test 1 and Test 2 by using one way ANOVA and Multiple Comparison Post hoc Tukey Test

ANOVA						
inflammation reduced						
	Sum of Squares	Df	Mean Square	F	Sig.	
Between Groups	9.345	3	3.115	5.114	.004	
Within Groups	31.671	52	.609			
Total	41.016	55				
Multiple Comparisons						
Dependent Variable: inflammation reduced						
Tukey HSD						
(I) treatment of drugs	(J) treatment of drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Standard	1.13786*	.29497	.002	.3550	1.9207
	test 1 (pulp)	.69143	.29497	.101	-.0915	1.4743
	test 2 (leaves extract)	.49357	.29497	.348	-.2893	1.2765
Standard	Control	-1.13786*	.29497	.002	-1.9207	-.3550
	test 1 (pulp)	-.44643	.29497	.437	-1.2293	.3365
	test 2 (leaves extract)	-.64429	.29497	.141	-1.4272	.1386
test 1 (pulp)	Control	-.69143	.29497	.101	-1.4743	.0915
	Standard	.44643	.29497	.437	-.3365	1.2293
	test 2 (leaves extract)	-.19786	.29497	.908	-.9807	.5850
test 2 (leaves extract)	Control	-.49357	.29497	.348	-1.2765	.2893
	Standard	.64429	.29497	.141	-.1386	1.4272
	test 1 (pulp)	.19786	.29497	.908	-.5850	.9807

*. The mean difference is significant at the 0.05 level

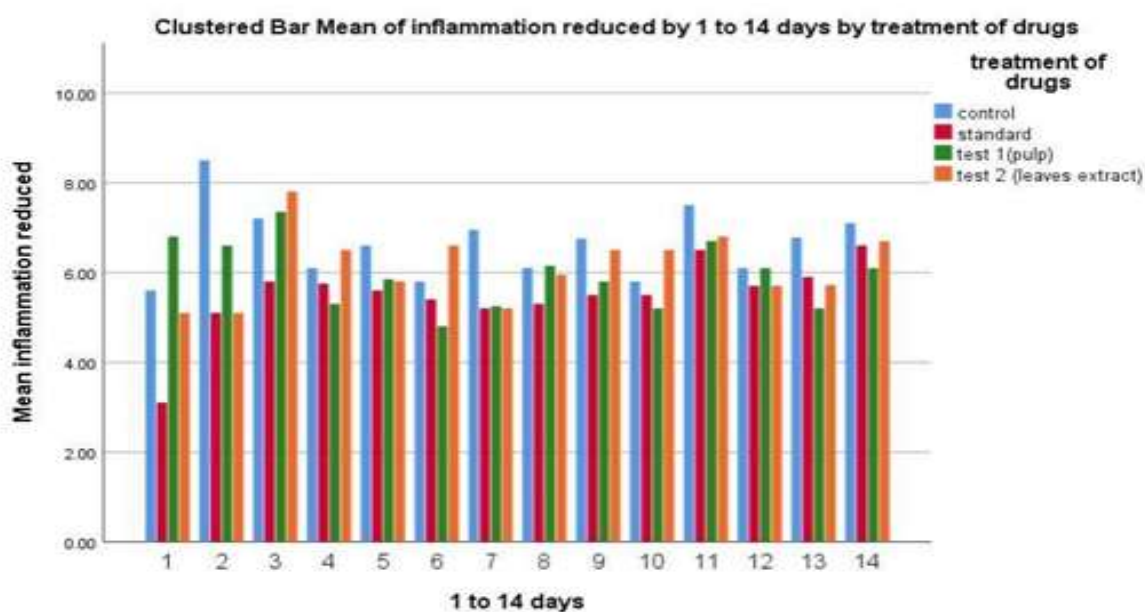


Figure 4: Illustrates the significant reduction in paw edema observed in the Standard, Test 1, and Test 2 groups compared to the Control.

The results highlights Java Plum's neuroprotective and anti-inflammatory effects. Quercetin, a flavonoid in Java Plum, has been shown to suppress neuro-inflammatory pathways and protect against oxidative stress. Additionally, tannins like ellagic acid in Java Plum is recognized for its anti-inflammatory and antioxidant aspects²¹. Java Plum's ability to regulate inflammatory pathways, particularly by inhibiting the NF-kB signaling cascade, may explain its neuroprotective effects. Anthocyanins, strong antioxidants responsible for Java Plum's colour, can suppress pro-inflammatory cytokines like TNF- α , IL-1 β , and IL-6, which are boosted in neuro-inflammatory conditions. By inhibiting this pathway, Java Plum may reduce inflammation linked to mood disorders and neurological diseases²².

Furthermore, although earlier studies examined the characteristics of pulp and leaf extracts separately, this study directly compares both the components using validated in vivo models for inflammation, anxiety, and depression, providing novel insight into their respective therapeutic potential.

The study assessed the anxiolytic and antidepressant effects of Java Plum extracts using the EPM and FST. EPM results showed increased time in open arms, suggesting reduced anxiety, while FST results revealed decreased immobility time, indicating potential antidepressant effects.

CONCLUSION

This study assessed *Syzygium cumini* (Java plum) extracts for their effects on depression, anxiety, and inflammation in rodents. Both pulp and leaf extracts showed anti-inflammatory effects, with the pulp extract also demonstrating anxiolytic and antidepressant properties. The leaf extract exhibited anti-inflammatory activity but lacked notable anxiolytic or antidepressant effects. These findings suggest Java Plum, especially its pulp, could be a potential treatment for mood disorders and neuroinflammation. More research is required to explore its long-term effects and mechanisms.

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Association of Caffeine Intake and Stress among University Students

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ABSTRACT

Objectives: The primary objective of the study was to determine the relationship between caffeine intake and stress among university students. While the secondary objective of the study was to find how the type and amount of caffeine consumption affects university students.

Methodology: A cross sectional study was conducted in which 154 undergraduate students of University of Management and Technology of ages 17 to 26 years were targeted. Questionnaires were administered using an interview-based technique, incorporating the Perceived Stress Scale—one of the most widely used psychological tools for assessing stress perception—and a measure to evaluate caffeine consumption by calculating the number of caffeine servings per week.

Results: The study highlighted the stress score according to which, 15% of the respondents had low intensity of stress, 66% had moderate intensity of stress, and 19% students were in the high stress category. We analyzed their caffeine consumption and the results indicated that 82% students had low consumption of caffeine, 14% were having moderate amounts of caffeine and only 4% students had high consumption of caffeine. Out of all caffeinated products, the most consumed caffeinated drink amongst the undergraduate male (WHY MALE) students of UMT was tea (43% responded 5-6 times/week), while coke/ carbonated beverages (38% responded 2-3 times/week) and milk chocolate bars (36% responded 4 times/week) followed at second and third place. The Pearson correlation coefficient between stress scores and caffeine intake is -0.007. This value is very close to zero, indicating almost no linear relationship between stress and caffeine

Conclusion: The study concluded that there is no relation between stress and caffeine intake.

Keywords: Anxiety, caffeine, caffeinated beverages, depression, stress

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INTRODUCTION

We live in a society where mental health problems are not recognized as issues requiring social change, leading to widespread nervousness, discomfort, and stress. In a society where education is increasingly emphasized, students face constant pressure, whether related to exams or basic assessments, which leads to deteriorating eating and drinking habits. As a result, students become more prone to unhealthy consumption patterns, particularly an increased intake of caffeine, with Pakistan being considered a country highly susceptible to excessive caffeine consumption²⁵.

National Institute of Health (NIH) describes stress as a feeling of emotional or physical tension that induces frustration, anger or nervousness. Stress can be induced due to any reason or any event that builds up tension, anxiety and frustration in one's body. It is a natural response of our body to a particular stressor^{1,2}. There are various factors that act as stressors in an undergrad's life such as academics, peers and social issues, any major changes or events such as leaving the university or getting a job, lack of sleep, financial issues and others. In any stressful conditions, our body releases a flood of hormones including adrenaline and cortisol. The release of these hormones increases heart rate and breathing rate, contracts muscles, raises blood pressure and sharpens senses. Stress also acts as a causative factor in many diseases, as it interacts with the disease and furthers worsens the disease. As a result of prolonged stress, our body starts showing different symptoms such as headache, aches and pains, heartache, insomnia, digestive problems, weakened immune system, depression, panic attacks, anxiety, sadness, and changing eating patterns^{3,4}.

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Caffeine, on the other hand, is a natural stimulant that blocks adenosine which is also a neurotransmitter that relaxes the brain and prevents tiredness. Its main effect is on the brain. It is naturally found in leaves and fruits of some plants. It is also present in coffee, green and black tea, soft drinks, energy drinks, cocoa chocolate bars, energy bars. Increased consumption of caffeine poses many negative health effect as it increases the release of cortisol in our body which is a hormone that is naturally released in our body in stress. This hormone further causes many negative effects such as increasing weight, endocrine diseases such as diabetes, cardiovascular diseases such as arrhythmias, mood swings and others.^{5,6} Approximately 80% of the world population consumes caffeine every day, with a daily intake of upto 200 mg, equivalent to around three espresso cups per day.⁷ According to FDA, healthy adults can consume caffeine up to 400mg/ day safely. Beyond this amount, caffeine can cause anxiety, difficulty in sleeping, addiction, heart problems, digestive problems, increased blood pressure, increased heart rate, and persistent fatigue and tiredness.⁸

Different people consume caffeine in different forms. Office workers, doctors, engineers, businessmen, merchants and homemakers consume caffeine to prevent tiredness and fatigue.⁹ Athletes and sports persons drink caffeine to enhance their performance whereas adolescents, teenagers and many adults, drink caffeinated beverages for their distinctive taste and the ability to boost energy²⁻⁴.

People consume caffeine in various ways such as coffee, tea, energy drinks, carbonated beverages, and chocolates. In Pakistan, tea is the most popularly consumed caffeinated drink in all age groups and nearly all members of households drink it as daily ritual. Majority of the people consume tea without knowing that it is a source of caffeine too. Surprisingly, increased consumption of chocolate exposes individuals to excess caffeine so toddlers and adolescents who consume chocolate as a healthy snack are actually imparting negative effects on their health. Another study revealed that intake of caffeine was causing anxiety and depression in secondary school children. These observed effects on mental health were distinct in children, being more dominant in male children¹⁰⁻¹².

The primary objective of the study was to determine the relationship between caffeine intake and stress among university students. While the secondary objective of the study was to find how the type and amount of caffeine consumption affects university students.

METHODOLOGY

IRB/ERC Approval:

The study was conducted after obtaining approval from the Research Ethics Committee of the University of Management & Technology, vide reference number RE-111-2023. Consent of the respondents was taken before taking them on board. The identity of the study participants was kept confidential and used solely for generating results of the research.

It was a cross sectional study. The study method was quantitative because the study correlates the relation between stress and caffeine consumption. The study used a non-probability sampling technique that is convenience sampling of 154 undergraduate students of University of Management & Technology, University of Health Sciences, University of Veterinary & Animal Sciences, University of South Asia.

Sample size was calculated to be 154 by using the formula and parameters given below.

$$n = [t^2 * p (1 - p)] / m$$

[Where n = sample size, t = confidence level - 95 % (1.96), p = Estimated prevalence – 88.5% (0.885) and m= Error -5% (0.05)]

The inclusion criteria included undergraduate students between ages 17 to 26 years of age and without any known illnesses

The Exclusion criteria included students below 17 years or having any illness or inborn errors Self-structured questionnaire was used for conducting online survey. It consisted of four parts:

1. Demographics
2. Perceived stress scale--American Sociological Association
3. Self-designed questionnaire
4. Food frequency questionnaire

Demographical part included Age, weight and height, a perceived stress score of American Sociological Association including questions that helped analyze people with low stress score between 0–13 points, moderate stress score between 14–26 points or high stress score between 27–40 points. Self-structured questionnaire included serving sizes of different products. The last section contained a questionnaire about food frequency for a week. This included questions regarding the frequency of the consumption of caffeinated products in particular serving sizes during the previous week which helped us analyze if the consumption of caffeine was low, moderate or high in

the respondents. Respondents were informed about the objective and they responded voluntarily. Data was collected online through Google Forms, respondents were reached through social media, and data was recorded on an MS Excel sheet.

In this study, SPSS V22 software was used for data analysis. The study applied bivariate correlation to find the relation between variables (stress and caffeine).

RESULTS

The study had 154 participants. The body mass index (BMI) of 61% participants was normal while 27% were overweight, and 12% were found to be grade-I obese. None of the participants was underweight.

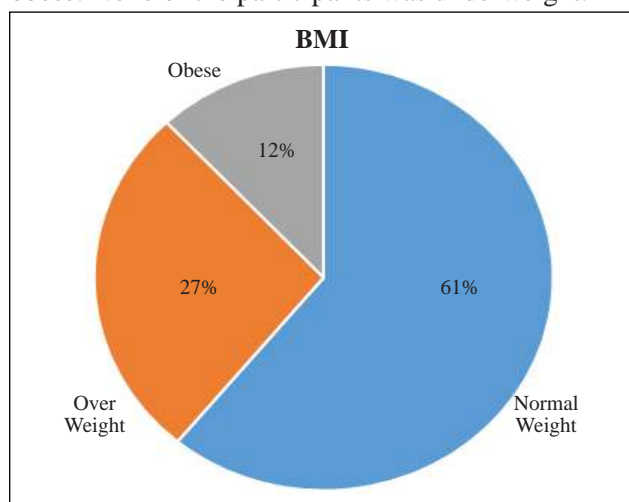


Figure 1: Percentages of students according to BMI (normal, overweight and obese)

Figure 2 shows that the stress score of 66% participants is moderate, 19% have high stress score, while 15% have low stress score. The scoring is based on Low= 0-13 (stress score), moderate 14-26 (stress score) and High=27-40 (stress score)

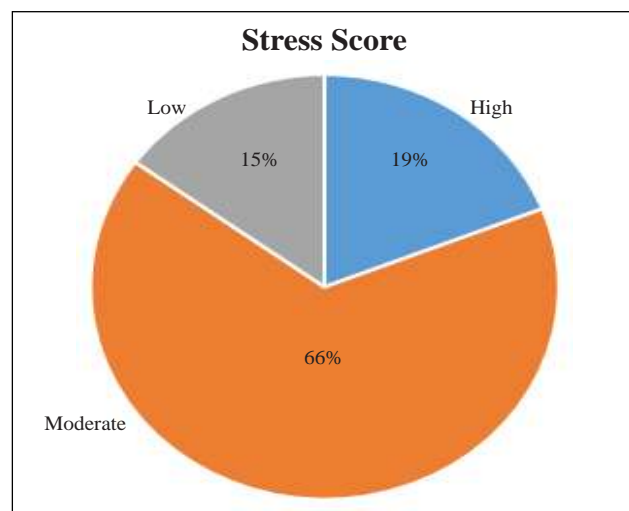


Figure 2: Percentages of students according to Stress Score (High, Moderate and Low)

Figure 3 represents weekly recall frequency of caffeinated products. According to this data, it can be interpreted that majority of participants had black tea (74.68%), green tea (50.65%), regular coffee/espresso (72.08%), instant coffee (68.83%), brewed coffee (81.1%) and filtered coffee (86.36%) 0 times/week, whereas the majority of participants had milk tea/chai (42.86%) 5-6 times/week.

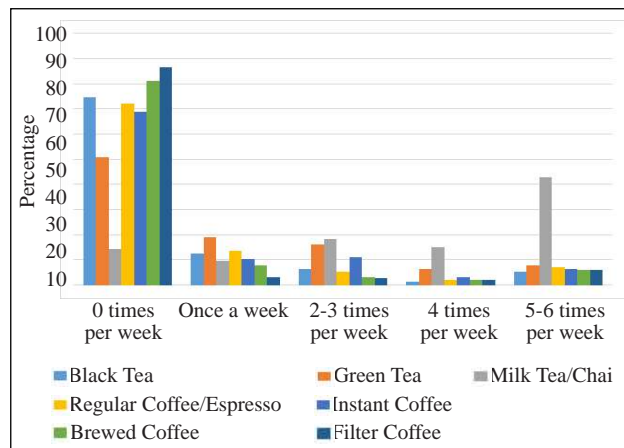


Figure 3: Caffeinated Beverages Intake in a Week

According to this data, 16.44% students had black tea, 32% students has green tea, 62.15% had milk tea/chai, 18.15% had espresso, 20.24% had instant coffee, 12.22% had brewed coffee, 8.87% had filter coffee, 19.16% had cold brewed coffee, 42.58% had coke, 19.81% had diet coke, 27.40% had sting, 20.12% had red bull, 24.81% had chocolate shake, 31.20% had milk chocolate and 15.18% had dark chocolate during the previous week. So, the consumption of milk tea/chai, coke and milk chocolate was more as compared to other caffeinated products.

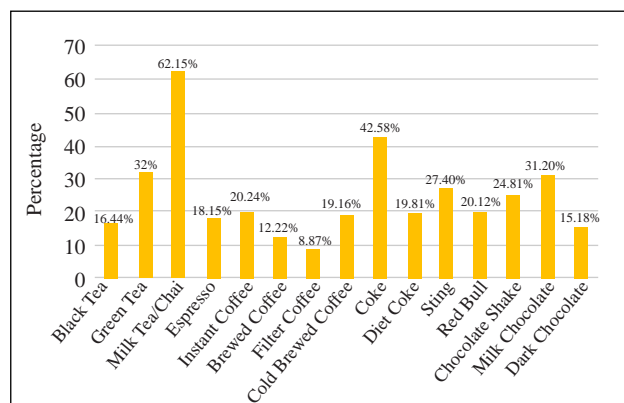


Figure 4: Frequencies of Caffeinated Product

The Pearson correlation coefficient between stress scores and caffeine intake is -0.007. This value is very close to zero, indicating almost no linear relationship between stress and caffeine intake in this dataset. The

significance (2-tailed) value for this correlation is 0.929. This p-value is much greater than the standard threshold of 0.05, meaning the correlation is not statistically significant. In other words, the observed relationship could easily be due to chance.

There is no meaningful or statistically significant relationship between stress scores and caffeine intake in this dataset.

The very weak negative correlation (-0.007) suggests that, if anything, higher caffeine intake is very slightly associated with lower stress, but this association is negligible and not statistically significant.

Table 1: Correlations Between Stress and Caffeine Intake

		Stress Score	Caffeine Intake
Stress Score	Pearson Correlation	1	-.007
	Sig. (2-tailed)	.929	.929
	N	154	154
Caffeine Intake	Pearson Correlation	-.007	1
	Sig. (2-tailed)	.929	.929
	N	154	154

DISCUSSION

Caffeine is the most widely used substance around the world. This study was conducted on different universities students (both private & government), wherein the majority reported its use in the form of coffee, tea, soda, and energy drinks. Caffeine consumption was found to be prevalent among students. This study analyzed the stress score in students, quantity and frequency of caffeinated products in the previous week as well as the relation between stress and caffeine consumption. Among 154; 29 students were classified to be in high stress, 102 were found to be in moderate stress and 23 students were in low stress (fig. 2). In a study of college students in the United States, total mean of caffeine consumption was 159mg/day for whole student population and total mean of regular students for both males and females was 173mg/day. Majority of the students were regularly consuming soda, coffee and tea¹¹. However, this study targeted students between the ages of 17 and 26 years and the total mean consumption of caffeine among students was 708.5 mg/week.¹² Majority of students were not consuming caffeinated products regularly. In the previous week, they were mostly consuming tea, coke, green tea and milk chocolate. As many as 62.15% students were consuming tea, 42.58% were consuming coke, 32% were consuming green tea and 31.2% were consuming milk chocolate.

In a survey from University of Lahore, Pakistan on caffeine intake among 185 students with 52 males students and 133 females, the benefits and hazardous effects of caffeine were examined.^{10,17} Products containing caffeine consumed by students were tea 62.4% and soft drinks (52.8%). Lowest consumption rate was observed for coffee 41.6%¹³. This study has some similarities with our study. In this study, 62.15% students were consuming tea and less than 50% were consuming coffee. The lowest consumption rate was that of filtered coffee 8.87%, brewed coffee 12.22%, dark chocolate 15.18%, and espresso 18.15% . Total 47.52% students were consuming energy drinks out of whom 27.4% were consuming Sting and 20.12% were consuming other energy drinks.

Another study suggested that caffeine consumption may be associated with stress but in this study there is no correlation between stress and caffeine consumption.¹² Another research found negative correlation between stress and caffeine.^{14,15} This study found no correlation between caffeine and stress. The study done by Conway did not report significant correlation between stress and caffeine consumption.¹³ Similarly Josue L et al also reported that there is no correlation between caffeine and stress that is also seen in our study. A research was conducted in Mumbai on consumption of caffeine which was associated with appetite and stress¹⁶⁻²². They found negative association of caffeine with appetite but no significant association with stress which is also substantiated by this study. Participants found caffeine to be helpful for its properties of helping in remaining wakeful, enhanced study hours, being able to concentrate, being better able to socialize as evident from the data given in the results. This could be due to the fact that caffeine does have some beneficial CNS stimulatory effects^{23,24}.

CONCLUSION

Through the analysis regarding caffeine consumption and stress, the study concluded that mostly students were consuming milk tea, cola drinks, and milk chocolate. Most of the students were found to be in moderate stress. Some were found to be in high stress, but their caffeine consumption was low and moderate. So, there is no significant relation of intake of caffeine in students with stress. Based on this data, caffeine intake does not appear to have a meaningful impact on stress levels.

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Authors' Contributions: **AN:** Designed the study, analyzed the data, wrote the manuscript, and approved the final version **FK:** Designed the study, analyzed the data, wrote the manuscript, and approved the final version **AT:** Designed the study and approved the final manuscript **SND:** Analyzed the data, wrote the manuscript, and approved the final manuscript **MT:** Collected the data and approved the final manuscript.

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