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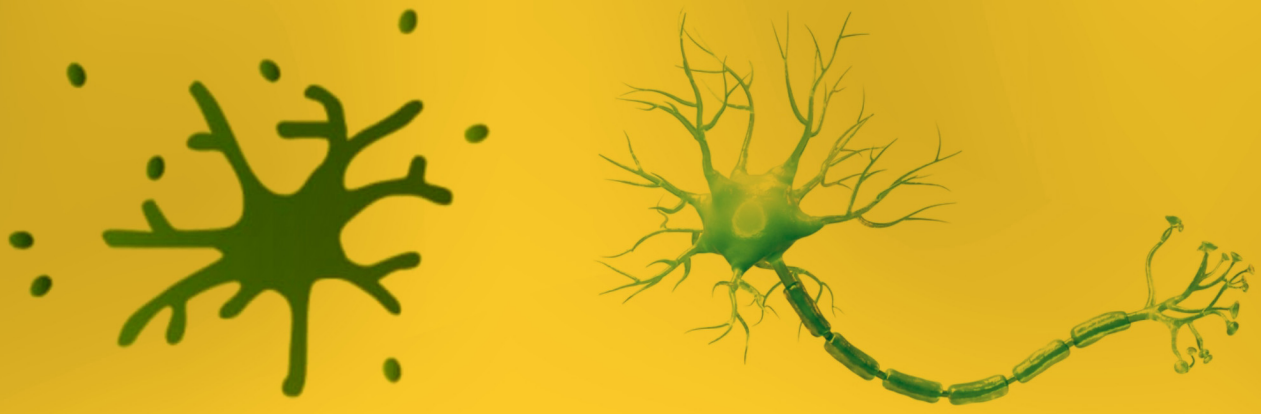

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RESEARCH ARTICLES

Bioscience Biotechnology Research Communications

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Editors Communique

Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

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Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

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Editor-in-Chief

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On the Prevalence and Distribution of Tobacco use and Evaluation of Awareness Levels Regarding its Detrimental Effects Among College Students in Riyadh, Saudi Arabia

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ABSTRACT

Tobacco use remains one of the most pressing public health challenges globally, and is the leading cause of preventable diseases, disabilities, and deaths, claiming more than eight million lives annually. Among these, nearly one and a half million deaths occur due to exposure to second hand smoke, highlighting the broader societal implications of tobacco use. In countries like Saudi Arabia, the youth population forms a substantial demographic, making them a critical group for public health interventions. The present questionnaire-based study attempts to analyse the prevalence and distribution of tobacco use and also has evaluated the awareness levels of the detrimental effects of smoking among college going students in Riyadh, Saudi Arabia. This study has found that 66.6% of participants were aware of the harmful effects of tobacco, 38.8% reported using tobacco in some form. Tobacco use was significantly higher among males, with 155 out of 289 males (53.63%) using tobacco, compared to 39 out of 149 females (26.17%). About 66.6% students demonstrated awareness of the harmful effects of tobacco use, while 33.4% lacked this knowledge. Among tobacco users, 53.6% believed shisha was less harmful than cigarettes, while 38.2% believed e-cigarettes posed minimal health risks. About 31.96% expressed willingness to quit, while 68.04% were not interested in quitting. The findings of this study have important implications for public health in Saudi Arabia. By identifying the prevalence and patterns of tobacco use among college students, it will provide valuable insights into the behaviors and attitudes driving tobacco consumption. Understanding the level of awareness regarding the harmful effects of tobacco will help in designing more effective educational campaigns that resonate with young adults.

KEY WORDS: Tobacco use, students, awareness levels, detrimental use

INTRODUCTION

Tobacco use remains one of the most pressing public health challenges globally. It is the leading cause of preventable diseases, disabilities, and deaths, claiming approximately 8 million lives annually. Among these, nearly 1.3 million deaths occur due to exposure to second hand smoke, (SHS) highlighting the broader societal implications of tobacco use. SHS kills approximately 1.2 million people per year, and 65,000 of these preventable deaths are seen in children under 15 years of age. Children with parents who are active smokers are nearly 70% more likely to attempt smoking by the age of 15, (Oberg et al 2011, Baha and Yildiz (2024).

According to the World Health Organization (WHO, 2021), tobacco consumption contributes significantly to the global burden of non-communicable diseases (NCDs), including cancer, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), and stroke. Despite extensive efforts to curb tobacco usage through policy interventions, public health campaigns, and legislation, its prevalence remains alarmingly high, particularly among youth and young adults, (Andhavarapu et al 2023).

The prevalence of tobacco use among youth is particularly concerning due to its long-term health implications. Studies have shown that individuals who start using tobacco at an early age are more likely to develop nicotine dependence, making it harder for them to quit later in life. The Global Youth Tobacco Survey (GYTS, 2017) reveals that a significant percentage of young people worldwide experiment with tobacco products during their teenage years, driven by factors such as peer pressure, stress, familial influence, and targeted marketing by the tobacco industry.

In countries like Saudi Arabia, the youth population forms a substantial demographic, making them a critical group for public health interventions. Research indicates that the Middle East has one of the fastest-growing rates of tobacco consumption globally. Cultural norms, social acceptance of smoking, and limited enforcement of anti-tobacco policies

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contribute to this trend. A study by Mandil et al. (2010) found that approximately 25-30% of Saudi university students were active smokers, with males being more likely to smoke than females. These figures underscore the urgency of addressing tobacco use among young adults in the region.

Saudi Arabia has made considerable strides in tobacco control, including implementing excise taxes on tobacco products, banning advertisements, and enforcing smoking bans in public spaces. However, the prevalence of tobacco use remains a significant concern, particularly among college students. According to a study by Almutairi (2016), nearly 21% of Saudi adults aged 15 and above reported using tobacco products, with the highest rates observed among young males. The study also highlighted the rising popularity of alternative tobacco products such as shisha (waterpipe) and e-cigarettes, particularly among the youth, as they are often perceived to be less harmful than traditional cigarettes.

The Riyadh region, as the capital and most populous city in Saudi Arabia, offers a diverse and representative population for studying tobacco use patterns. College students in Riyadh, who are transitioning into adulthood, face unique pressures that may contribute to tobacco initiation and continuation. Factors such as academic stress, financial independence, social networking, and exposure to diverse cultural norms make this group particularly vulnerable to tobacco use. Awareness regarding the detrimental effects of tobacco use is a critical determinant of its prevalence. Numerous studies have established that higher levels of awareness are associated with reduced tobacco initiation and increased cessation rates. For instance, the WHO's Framework Convention on Tobacco Control (FCTC) emphasizes the importance of public education campaigns in reducing tobacco use. However, despite widespread knowledge about the health risks associated with smoking, there exists a significant gap in the understanding of its broader consequences, including environmental and economic impacts.

In Saudi Arabia, awareness campaigns such as anti-smoking clinics and public health initiatives aim to educate the population about the dangers of tobacco. Yet, evidence suggests that these efforts often fail to reach college students effectively. A study conducted by Al-Zalabani et al. (2015) revealed that while most university students were aware of the general health risks of smoking, many were unaware of its specific associations with diseases like oral cancer, infertility, and chronic respiratory conditions. This gap in knowledge underscores the need for targeted interventions that resonate with young adults. Understanding the sociocultural and behavioral factors that influence tobacco use is essential for designing effective prevention and cessation strategies.

In Saudi Arabia, smoking is often viewed as a social activity, with gatherings frequently involving shisha or cigarette smoking. This normalization of smoking in social settings poses a significant barrier to cessation efforts. Moreover, the perception of shisha as a less harmful alternative to cigarettes has led to its increasing popularity among young adults, particularly college students.

Behavioral factors such as stress, peer influence, and exposure to smoking among family members also play a crucial role in tobacco initiation and continuation. Academic stress is a significant factor for college students, who may turn to smoking as a coping mechanism. Peer influence is another critical driver, as individuals are more likely to smoke if their friends or social circles endorse or participate in the habit. Family dynamics also play a role; exposure to tobacco use within the family can normalize the behavior, making young individuals more susceptible to trying tobacco products.

In recent years, the advent of e-cigarettes and other alternative tobacco products has added a new dimension to the tobacco use landscape. Marketed as safer alternatives to traditional cigarettes, these products have gained significant traction among young adults worldwide, including in Saudi Arabia. Studies have shown that many users of e-cigarettes are unaware of their potential health risks, such as exposure to harmful chemicals and an increased likelihood of transitioning to combustible tobacco products. In a study by Awan et al. (2020), the use of e-cigarettes among Saudi college students was reported to be on the rise, with many students perceiving them as a trendy and less harmful option. The growing popularity of such products highlights the importance of comprehensive tobacco control strategies that address both traditional and alternative forms of tobacco. Public health campaigns must focus on dispelling myths surrounding the safety of e-cigarettes and educate young adults about their associated risks. Saudi Arabia's government has implemented several tobacco control measures aligned with the WHO FCTC (2021).

These include increasing taxes on tobacco products, banning smoking in public places, and introducing plain packaging regulations. However, the effectiveness of these policies among college-going students has been limited. One reason for this is the lack of targeted strategies addressing the specific needs and behaviors of this demographic. For instance, while smoking bans in public places are effective in reducing second hand smoke exposure, they may not deter students who smoke in private or social settings. Similarly, tax increases may not significantly impact students from affluent families or those who switch to cheaper alternatives like shisha or bidis. Tailored interventions, such as campus-based anti-smoking campaigns and peer-led education programs, could be more effective in addressing tobacco use among college students.

This study aims to address critical gaps in understanding the prevalence and distribution of tobacco use among college-going students in the Riyadh region of Saudi Arabia. The findings of this study have important implications for public health in Saudi Arabia. By identifying the prevalence and patterns of tobacco use among college students, it will provide valuable insights into the behaviors and attitudes driving tobacco consumption. Understanding the level of

awareness regarding the harmful effects of tobacco will help in designing more effective educational campaigns that resonate with young adults.

Methodology

Study Design: This research employed a cross-sectional study design to assess the prevalence and patterns of tobacco use and evaluate awareness regarding its harmful effects among college-going students in the Riyadh region of Saudi Arabia. This design was chosen to capture a snapshot of the population at a specific point in time and efficiently examine relationships between demographic factors, tobacco use patterns, and awareness levels.

Study Population: The study targeted college-going students aged 18–25 years, enrolled in various universities and colleges across Riyadh. This population was selected because they represent a critical demographic for tobacco prevention efforts, given their susceptibility to peer influence and stress-induced behaviors.

Sample Size: The study included 500 participants, deemed an appropriate sample size to ensure statistical reliability and validity of findings. This sample size aligned with prior research examining similar populations and allowed for meaningful subgroup analysis.

Sampling Method: A simple random sampling technique was employed to select participants from the student population. This method ensured that each individual in the target population had an equal chance of being included, minimizing selection bias.

Inclusion Criteria: Students included 18–25 years, both male and female, enrolled in colleges and universities in Riyadh. Students who provided informed consent to participate in the study.

Exclusion Criteria: Students below 18 or above 25 years of age. Individuals who declined to provide informed consent. Students with prior diagnosed smoking-related illnesses, as this could skew perceptions and awareness data.

Data Collection: Data were collected using a structured questionnaire, developed based on prior studies and validated for use in similar populations. The questionnaire was administered in both English and Arabic to ensure accessibility and comprehensibility. It included the following

sections: Demographics: Age, gender, educational level, and socioeconomic background. Tobacco Use Patterns: Current and past tobacco use (smoking, smokeless, or both). Types of tobacco products used (e.g., cigarettes, shisha, e-cigarettes).Frequency and duration of use. Reasons for initiation and continuation of tobacco use.

Awareness and Knowledge: Awareness of the health risks associated with tobacco use. Sources of information (e.g., media, family, peers). Perceptions of shisha and e-cigarettes as less harmful alternatives. Willingness to Quit: Intentions and attempts to quit tobacco use. Barriers to quitting.

Data Collection Process Pre-Study Preparation: Ethical approval was obtained from relevant institutional review boards (IRBs). Data collectors were trained on the study's objectives, ethical considerations, and data collection

procedures. Recruitment: Students from randomly selected colleges in Riyadh were approached. The study's objectives and procedures were explained to potential participants. Written informed consent was obtained before participation. Survey Administration:

The structured questionnaire was administered either face-to-face or electronically, based on participant preference. Privacy and confidentiality were ensured during data collection to encourage honest responses. Data Quality Assurance: A pilot study was conducted with 10% of the sample size to test the questionnaire for clarity and consistency. Any issues identified during the pilot study were addressed before full-scale data collection.

Ethical Considerations: Informed Consent: Participants were fully informed about the study's objectives, procedures, and their rights, including the option to withdraw at any time without repercussions. Confidentiality: Personal identifiers were removed from the dataset to ensure anonymity. Approval: Ethical clearance was obtained from an institutional ethics review board. Voluntary Participation: Participation was entirely voluntary, with no incentives provided to avoid coercion.

Data Analysis: Descriptive Statistics: Demographic characteristics, prevalence, and patterns of tobacco use were summarized. Data on awareness and willingness to quit were presented using means, medians, and percentages. Inferential Statistics: Chi-square tests were used to identify associations between demographic factors (e.g., age, gender) and tobacco use patterns. Logistic regression analyses were conducted to explore predictors of tobacco use and awareness levels. Software: Statistical analyses were performed using SPSS or a similar software package to ensure accuracy and reliability.

Outcomes: This methodology provided comprehensive insights into: The prevalence and distribution of tobacco use among college students in Riyadh. Awareness levels regarding the harmful effects of tobacco. Key factors influencing tobacco use and cessation willingness.

RESULTS AND DISCUSSION

The study included 500 college-going students aged 18–25 years from various colleges in the Riyadh region. Among the participants, 289 (57.8%) were male, and 211 (42.2%) were female. The age distribution was as follows: 162 participants (32.4%) were aged 18–20 years, 189 participants (37.8%) were aged 21–23 years, and 149 participants (29.8%) were aged 24–25 years. Out of the 500 participants, 194 (38.8%) reported using tobacco in some form. Tobacco use was

Table 1: Demographic Distribution

Category	Group	Number of Subjects	%
Gender	Male	289	57.8 %
Gender	Female	211	42.2 %
Age Group	18-20 years	162	32.4 %
Age Group	21-23 years	189	37.8 %
Age Group	24-25 years	149	29.8%
Tobacco Usage (Gender Wise)	Male	155	53.63%
Tobacco Usage (Gender Wise)	Female	39	26.17%

Figure 1: Demographic Characteristics

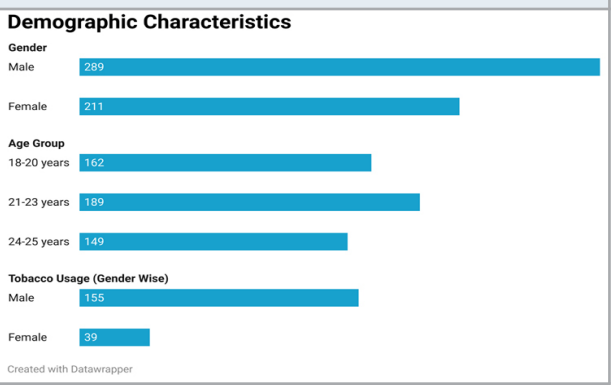


Table 2: Type of Tobacco used

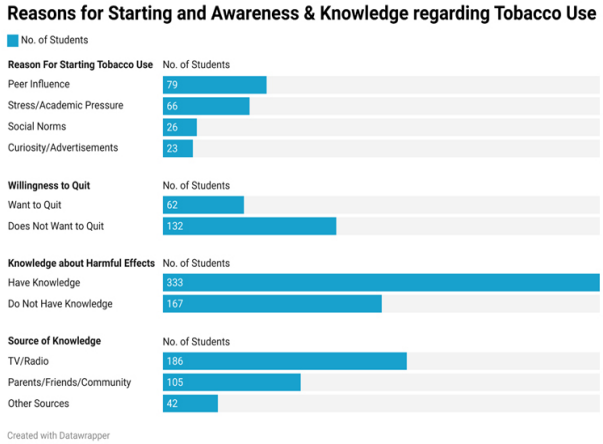
Type of Tobacco	Number	Percentage
Cigarettes	93	18.6
Shisha	54	10.8
Smokeless Tobacco	35	7
Dual Users	12	2.4
Total	194	100

significantly higher among males, with 155 out of 289 males (53.63%) using tobacco, compared to 39 out of 149 females (26.17%). (Table 1)

Of the total 194 tobacco users, 93 participants (18.6%) smoked cigarettes while 54 smoked shisha (10.8%). 35 participants (7%) used smokeless tobacco products while 12 participants (2.4%) reported using both smoked and smokeless tobacco products.

Of the 500 participants, 333 (66.6%) demonstrated awareness of the harmful effects of tobacco use, while 167 (33.4%) lacked this knowledge. Among those who were aware, 186 participants (55.85%) cited television or radio as their primary source of information, 105 participants (31.53%) mentioned family or friends as their source of knowledge while 42 participants (12.61%) learned about tobacco risks through social media or other platforms. Despite the awareness, a substantial proportion of participants underestimated the risks associated with shisha and e-cigarettes.

Figure 2: Knowledge and Awareness of Tobacco Use



Among tobacco users, 53.6% believed shisha was less harmful than cigarettes, while 38.2% believed e-cigarettes posed minimal health risks. Participants reported various reasons for initiating tobacco use. 79 participants (40.72%) cited peer influence as the primary reason. 34.02% identified stress or academic pressure while 13.4% mentioned social norms and cultural acceptance with 11.86% reporting being influenced by advertisements or curiosity.Among the 194 tobacco users, 62 participants (31.96%) expressed a willingness to quit, while 132 participants (68.04%) were not interested in quitting. 90 participants (64%) cited addiction as the main obstacle. 30 participants (21%) reported a lack of cessation support. 20 participants (15%) mentioned social influences as a barrier.

Statistical Analysis: A chi-square test showed a significant association between gender and tobacco use patterns ($\chi^2 = 45.31$, $p < 0.001$), with males being more likely to use tobacco than females. Logistic regression analysis indicated that participants with higher awareness levels were 1.8 times more likely to avoid tobacco use ($p < 0.05$). No significant association was found between age group and tobacco use ($\chi^2 = 3.21$, $p = 0.201$).

The study investigated the prevalence, types, and factors influencing tobacco use among college-going students in

Riyadh, Saudi Arabia, and the findings reveal important patterns and gaps. The prevalence of tobacco use in this study was 38.8%, with significantly higher rates among males (53.63%) than females (26.17%). This is consistent with findings from Mandil et al. (2010), who reported a prevalence of 25-30% among Saudi university students, with higher rates among males. Similarly, Almutairi (2016) found that 21% of Saudi adults used tobacco, with males being the dominant users. These gender-based disparities reflect cultural norms and social acceptability of smoking among men in the region.

Table 3: Gender and Tobacco Use

Gender	Tobacco Users	Non-Tobacco Users	Total
Male	155	134	289
Female	39	172	211
Total	194	306	500

Chi-Square Value: 61.98 p-value: 3.46×10−15 (significant at p<0.05)

Table 4: Age Group and Tobacco Use

Age Groups	Tobacco Users	Non-Tobacco Users	Total
18-20 years	84	78	162
21-23 years	70	117	189
24-25 years	40	111	149
Total	194	306	500

•Chi-Square Value: 21.41p-value: 2.25×10−5 (significant at p<0.05p)

The prevalence in Riyadh also echoes findings from the Global Youth Tobacco Survey (GYTS, 2021), which reported a global tobacco use prevalence of 37.7% among youth, with males consistently outpacing females. The slightly higher rates observed in this study may be attributed to the regional popularity of shisha and the growing accessibility of alternative tobacco products like e-cigarettes.The significant association between gender and tobacco use ($\chi^2 = 61.98$, $p < 0.001$) supports the findings of Al-Zalabani et al. (2015), who highlighted the gendered patterns of smoking in Saudi Arabia. Smoking among females in this study was 26.17%, higher than the 6-20% range reported in earlier studies (Almutairi, 2016; Awan et al., 2020). This may indicate a shift in social norms or the influence of targeted marketing of "socially acceptable" products like shisha and e-cigarettes to women, as highlighted by Warren et al. (2008).

Globally, similar trends have been noted. A study by Baska et al. (2012) found a prevalence of 20% among European female university students, suggesting that while cultural barriers limit female smoking in conservative societies, urbanization and globalization are bridging this gap.The predominance of cigarettes (18.6%) and shisha (10.8%) in this study aligns with findings from Shaikh et al. (2022), who observed a regional shift from traditional tobacco to socially endorsed forms like shisha. Awan et al. (2020) noted that 60% of young adults in Saudi Arabia perceived shisha as less harmful than cigarettes, a misconception echoed in this study, where 53.6% of users believed shisha posed fewer health risks. The use of smokeless tobacco (7%) was slightly lower than the 10-15% reported in South Asian studies (Gupta et al., 2010; Sridharan, 2014).

This may reflect regional preferences, as smokeless products like gutkha are more popular in countries like India and Pakistan. This study found that 66.6% of participants were aware of the harmful effects of tobacco, consistent with Zahiruddin et al. (2011), who reported a 68% awareness rate among Indian youth. However, misconceptions about shisha and e-cigarettes persist, underscoring the need for more focused education campaigns.The influence of media (TV/radio: 55.85%) as a source of awareness was consistent with findings from Hemagiri et al. (2011), who highlighted the role of mass media in shaping perceptions about tobacco. Social media, cited by 12.61% of participants, is emerging as a critical platform for tobacco awareness, a trend supported by studies like Alghamdi et al. (2022), which emphasized the role of digital campaigns in youth engagement.

The primary reasons for tobacco initiation in this study—peer influence (40.72%), stress (34.02%), and social norms (13.4%)—align with findings from Mandil et al. (2010) and Garg et al. (2012), who reported peer pressure and academic stress as significant factors. This highlights the universal nature of these drivers across different cultural contexts. Interestingly, the role of advertisements and curiosity (11.86%) was lower than the 20-30% reported by Tranby et al. (2022) in Western contexts. This may reflect stricter advertising regulations in Saudi Arabia, though indirect marketing through social media remains a concern.

Among tobacco users, 31.96% expressed a willingness to quit, similar to the 30-35% reported by Zahiruddin et al. (2011) and Garg et al. (2012). The primary barriers to quitting—addiction (64%), lack of cessation support (21%), and social influences (15%)—mirror findings from Golechha (2016), who emphasized the need for accessible cessation programs and societal support systems. The significant association between gender and tobacco use ($\chi^2 = 61.98$, $p < 0.001$) supports global trends reported in studies like Warren et al. (2008) and Mandil et al. (2010). However, the lack of a significant association between age and tobacco use ($\chi^2 = 3.21$, $p = 0.201$) diverges from studies like Al-Zalabani et al. (2015), who found higher prevalence among older youth. This may be due to the limited age range in this study (18–25 years), which captures a relatively homogenous population.

CONCLUSION

This study found that 66.6% of participants were aware of the harmful effects of tobacco, 38.8% reported using tobacco in some form. Tobacco use was significantly higher among males, with 155 out of 289 males (53.63%) using tobacco, compared to 39 out of 149 females (26.17%).About, 66.6% demonstrated awareness of the harmful effects of tobacco use, while 33.4% lacked this knowledge. Among tobacco users, 53.6% believed shisha was less harmful than cigarettes, while 38.2% believed e-cigarettes posed minimal health risks. About 31.96% expressed willingness to quit, while 68.04% were not interested in quitting. The findings of this study have important implications for public health in Saudi Arabia. By identifying the prevalence and patterns of tobacco use among college students, it will provide valuable insights into the behaviors and attitudes driving tobacco consumption. Understanding the level of awareness regarding the harmful effects of tobacco will help in designing more effective educational campaigns that resonate with young adults.

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Approval: Ethical clearance was obtained from an institutional ethics review board.

Conflict of Interest statement: Author declares no conflict of interest

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Data Availability: Data are available and can be shared on reasonable request

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Ameliorative Effects of Banana in florescence *Musa sp* to Mitigate the impact of $CuSO_4$ on *Channa punctatus*

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ABSTRACT

Copper is highly toxic to fish, leading to harmful effects such as cell damage, genetic mutations, and cancer In aquatic environment it affects both human health and the aquatic ecosystem. Accumulation of copper sulphate on blood and tissue causes changes in the liver and kidney of *Channa punctatus*. Three sub-lethal concentrations of $CuSO_4$ (0 .45 mg/l, 0.72 mg/l, and 1.2 mg/l) were taken, One group with pretreatment of BFE exposed to $CuSO_4$, another group exposed to highest dose of $CuSO_4$ were treated BFE. Changes noted at intervals of 10, 20, and 30 days of blood , and tissues samples in haematology and histological parameters. In chemically treated groups. A decrease in RBC and Hb counts ,increase in WBC counts , and some nuclear abnormalities were observed Improvement in blood parameters noted in fish treated with BFE. Chronological histopathological damage, including swelling of hepatic cells, hepatocellular necrosis, vacuolization, inflammation, and hepatic cell damage observed in fish exposed to $CuSO_4$. In BFE treatment, improvements of vital organs were noted, with decreased inflammation, necrosis, and vacuolation in hepatic and renal structures. The banana inflorescence extract can be used as antioxidants to counteract the damage induced by copper sulphate in *C. punctatus*.

KEY WORDS: *CHANNA PUNTATUS*, CUSO4, NECROSIS, VACUOLATION, NUCLEAR ABNORMALITIES,

INTRODUCTION

Heavy metals are natural environmental components and large quantities of these heavy metals are accumulated as a result of land- based activities in the aquatic ecosystem (Javed et al. 2017). Nowadays, heavy metal residues have become a serious concern because of their continuous increase in air and aquatic environment (Abah et al. 2016; Javed and Usmani 2019). Increased human population with their anthropogenic activities, both underground and surface water supplies are now affected with the heavy metals resulting in depletion of the aquatic organisms (Waqar et al. 2013 Vasconselos et al 2024).

The contamination of aquatic ecosystem with heavy metals is regarded to be dangerous not only for aquatic fauna but also for the human as the consumer of fish for food, (Sabullah et al. 2015). At present, the aquatic pollution has increased many times due to the introduction of modern technologies using heavy metals as raw materials for different functions (Wong et al. 2001, Wu et al 2019). The adverse impacts of these pollutants on aquatic ecosystems and human health emphasize the need for effective pollution control measures, proper waste management, and sustainable practices to protect and preserve water quality (Perkumien et al., 2023).

Among metals, copper is one of the essential trace elements that plays an important role in the growth and development of organisms. At the same time, Cu is also one of the most poisonous metals that affects aquatic species and ecosystem (Singh et al. 2010). Heavy metal in relatively high concentrations and excessive use can result in toxic metal complexes that build in water, creating biological system imbalances and results in oxidative stress, (Lushchak 2011,Vasconselos et al 2024).

Apparently, the demand for copper continues to increase annually as it is used in water pipelines, intelligent houses and buildings, electrical motors, power lines, electrical appliances, healthcare, environment-related industries, computers and communication devices. This has further increased the use of copper in industry, while increasing copper contamination of the environment (Wani et al., 2020).

Heavy metals accumulate mostly in water bodies, affects a wide variety of aquatic organisms. In recent years, chemical biomonitoring and histopathology have often been combined with the evaluation of biomarkers representing early indicators of biological effects *Chana punctatus* is one of

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the most widely distributed freshwater fish and 26 species discovered in Asia, North eastern India gives home to the nine species of *Channa* (Vishwanath and Geetakumari, 2009). Eight species belonging to *C. barca and* and *C. bleheri*) are found all over India). Considering *Channa punctatus* and adaptable, it has It is taken as least concern by the IUCN red list threatened data book (IUCN 2014).

According to El-Moselhy et a (2014), metal bioaccumulation by fish is subsequently accumulated in specific organs and later distributed to different organs such as the liver, kidney, gills, heart, bone, brain and digestive tract. Fish has the ability to easily accumulate toxicants in their organs and cellular functions during exposure (Ajani and Akpoilih 2010). Thus, the effect of accumulated toxicants will cause severe damage to the piscine systems, leading to an overall toxic effect of metabolic reactions as well as physical and behavioural activities.

Haematological studies are useful in assessing the health of fish subjected to changing environmental conditions. Fish blood is being studied as an indicator of pathological changes and environmental monitoring system (Walter et al. 2006). Therefore, it is important to diagnose the functional and systemic state of fish exposed to toxicants.Metals can cause alteration in haematological indices in fish. Increase or decrease in blood parameters is considered to be the symbol of unhealthy state, environmental stress, or tissue injury (Hassan et al. 2018). The histopathological changes due to exposure of heavy metals are helpful in evaluating their toxic effects in different species of fishes (Clemente et al. 2013 Chakraborty and Sarkar 2023).

The liver of fish exposed to heavy metals showed congestion of the central vein, edema, and nuclear pyknosis and degeneration in hepatic cells of different species (Kaoud and El-Dahshan 2010; The histological alterations due to heavy metals in the intestines of fish include atrophy in the muscularis, necrotic changes in the intestinal mucosa, and sub-mucosa with degenerative cells in the intestinal lumen (Padrilah et al. 2018). Thus, the current study was planned to investigate the effect of induced toxicity of Copper Sulphate on the haematology and the histolopathology of *Channa punctatus*.

Many researchers' study to neutralize the toxicity induced by heavy metals by using naturally found minerals and vitamins. Liu et al. (2023) in their study, the anti-inflammatory and antioxidant activities of the tocotrienol-rich fraction from rice bran oil and its potential mechanism were verified in a zebrafish $CuSO_4$ inflammation model. According to a study conducted by China et al. (2010), banana flower extract is a potential natural source of antioxidants that might help reduce oxidative stress by lowering hepatic cell damage caused by free radicals in the iron-mediated Fenton reaction. The anti-cancer activities and anti-proliferative effects were also assessed.

The current study selected banana inflorescence extract to explore its potential in protecting freshwater fish from copper poisoning. This research focuses on the haematological response to chemical treatment with $CuSO_4$, emphasizing blood analysis. Additionally, the study examined the histological structure of key organs like the liver and kidney, due to their crucial roles in metabolizing various chemicals and eliminating cytotoxic and metabolic byproducts. The liver and kidneys are essential in processing toxic substances, meaning no chemical is entirely safe from potentially harming these organs. As a result, liver and kidney damage often serve as the first indication of toxicity. Thus, research over the past few decades has aimed to identify compounds that can mitigate these harmful effects and facilitate recovery from the damage caused by toxins. However, the protective role of banana inflorescence extract against the degree of toxicity of copper sulphate are not well documented.

MATERIAL AND METHODS

Collection of fish: *Channa punctatus*, ranging in length from 13 to 15 cm and weighing between 24 and 30 grams, were procured from a nearby market. To eliminate external fungal and algal infections and prevent potential skin issues, the fish underwent thorough washing in tap water followed by treatment with a 0.02% potassium permanganate (KMNO4) solution. They were then placed in aerated dechlorinated water under laboratory conditions, maintaining a natural photoperiod of 12 hours light and12 hours dark, and a temperature of 26°C for a 15-day acclimation period prior to the commencement of the experiment. Throughout this period, the fish were fed twice daily with "Optimum" artificial fish food from Perfect Companion Group Co., Ltd., Thailand, and daily maintenance included the removal of waste materials and uneaten feed to ensure optimal fish health.

Experimental design and specimen treatment: The selection of healthy fish for copper exposure were divided Karber Method into seven groups The LC_{50} was determined (Hamilton et al., 1977). Group I served as the control, while groups II, III, and IV were exposed to different sublethal concentrations of copper sulphate (0.45 mg/l, 0.72 mg/l, & 1.20 mg/L) for 10, 20, and 30 days, respectively. Groups V received treatment with banana flower extract (BFE), while group VI were chemical pretreated BFE followed by 1.20 mg/l of $CuSO_4$ exposure and in contrast other groups were first exposed to 1.20 mg/l $CuSO_4$ followed BFE treatment. Each treatment was performed in triplicate).Copper sulphate, formalin, ethanol, KMnO4, methanol, Giemsa 5% solution, haematoxylin, eosin are used during the experiment from the Zoology Lab, Royal School of life Science. Fresh banana (Musa sp.) inflorescence were purchased at the neighbourhood market, cleaned, sliced, dried at 40 degrees Celsius, powdered, and kept at 4 degrees Celsius.

Diet preparation: A total of 2 kg of banana inflorescence powder was extracted and processed using the decoction method. The resulting extract was then combined with 0.5 kg of salt (NaCl) and 1.0 kg of "Optimum" fish feed powder at a ratio of 10:1. Sterilized water was added as needed to form a dough, which was then used to create pellet feed. These pellets were dried in an oven at a temperature of 40°C and stored in an airtight container. The experimental

diets were fed to the fish at a rate of 3% of their live body weight, with the daily ration split into two equal meals at 8:45 am and 2:45 pm over a period of 2 weeks.

Sample Collection: Six samples of blood were taken from every group of fish Whole blood count parameters were determined using EDTA- containing blood. cells. Blood. Smear was fixed with absolute methanol, air dried and room temperature and counterstained with 5 and studied under the light microscope.

Total Count (TC) RBC (Erythrocyte): Erythrocytes were counted in the hemocytometry chamber and expressed as 10^6 cells per mm^3, following Wintrobe's (1967) methodology. Counting was done within five smaller squares: the 1st, 5th, 13th, 21st, and 25th

WBC (Leucocyte): Blood samples were diluted 1:20 with Turk's diluting solution before being put onto the haemocytomer. The four huge (1 square millimetre) corner squares of the haemocytomer were inspected under a microscope. Cells that touched the boundary . The total number of white blood cells was determined and expressed as cells per cubic millimetre using the method outlined by Wintrobe (1967).

Estimation of haemoglobin: A haemometer tube was filled with 10 drops of N/10 hydrochloric acid (HCl). A pipette was used to extract blood up to 20 cm, which was then discharged into the tube, mixed, and allowed to stand for 30 minutes. The tube was gradually filled with distilled water until it matched the colour of the

Statistical Analysis: Experiments were conducted in triplicates. The data observed in the experiment were statistically analyzed on One way ANOVA for individual group wise comparison was administered for testing the hypothesis. The data shown are the average of three replicates + SE and statistical significance was tested at $p<0.001$(***), $p<0.01$(**),& $p<0.05$(*).

Histopathological studies: The selected fish were euthanized and organs such as the liver and kidney were removed and fixed in 10% formalin for 48 hours and dehydration using various concentrations of ethanol (50%, 70%, 90%, and 100%) and were for kept for 20-30 mins each time. The tissues were then cleaned in Xylene for 30 minutes, immersed in liquid paraffin wax heated to 60°C, and embedded in blocks. A rotatory microtome was utilized to cut the sample blocks into slices that were 5 µm thick. These sections were then , flattened on a hot plate, and stained with hematoxylin for one minute followed by counterstaining with eosin for two minutes. After appropriate preparation, the sections were mounted using DPX. Images were captured using an oil immersion compound microscope with a 100X objective lens magnification.

Table 1.1 Behavioral changes and morphological deformities of *Channa punctatus* upon exposure to different concentration of $CuSO_4$.

Observation	Control	Pretreatment with BFE	Exposing pretreated fishes to $CuSO_4$	Effects of CuSO4	Recovery response after treated with BFE
Swimming Pattern	R	R	S	S	S
Scale loss	-	-	*	***	**
Head lesion	-	-	+	+++	++
Fin and tail movement	A	A	A	In	In
Discolouration of fish	N	N	*	***	*
Excretion	+	+	++		++
Mucus secretion	-	-	+	+++	++

R, relax; S, at the surface of the water; Iv, Inverted; A, active; In, inactive; N. normal; *, little loss; **, moderate loss; +, little; ++, moderate; +++, high; -. nil.

RESULTS AND DISCUSSION

The behavioural changes and deformities included alterations in swimming patterns, food intake, and excretions from the fish, with severity increasing in correlation with higher $CuSO_4$ concentrations the fish became more lethargic, exhibited reduced activity, and lost their healthy appearance and vigour.

Table 1.1 shows the observation of the behavioural changes of *C. punctatus* treated with $CuSO_4$. Upon $CuSO_4$ exposure, *C. punctatus* was recorded to change its swimming pattern as the fish tend to swim erratically with signs of suffocation at the water surface. Swimming pattern changes is one of the obvious signs of chemical exposure onto fish regardless of the exposure routes either by oral exposure or by a flow-through system. Table 1.1 shows the *C. punctatus* swimming style, which has changed to

become inverted and the fish tend to swim at the surface of the water upon increasing $CuSO_4$ concentrations in the water. The behavioural changes and deformities observed are in accordance with a previous study by Sabullah and Khayat (2015) on *Puntius schwanenfeldii*. After thorough observation, the fishes in the BFE treated groups were seen improving in their motility such as increased in their fins and tail movement as well as their swimming behaviour.

Figure 1: Photos taken during experimental period I, control; head lesion (II), & upward swimming (III); body lesion (IV), & scale loss and discoloration (V)

Figure 2 Haematological parameters of Channa punctatus with control and after giving BFE for 30 days 30 days experimental period.

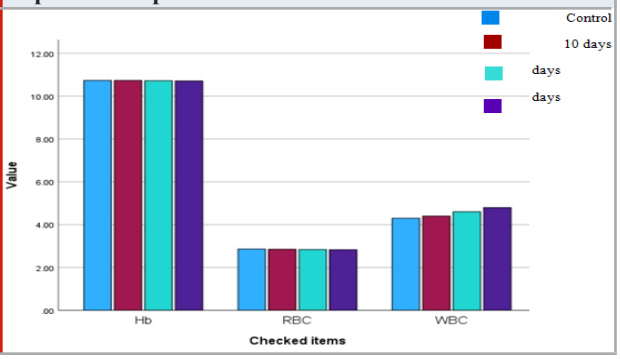
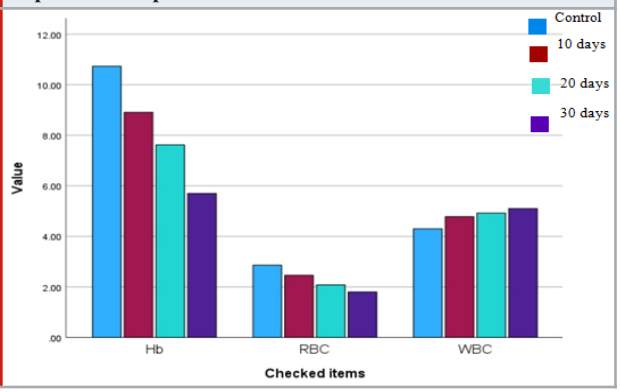


Figure 3 Haematological parameters of Channa punctatus with control and after exposing to CuSO4 for 30 days experimental period.



Excretion and mucus secretion: In response to $CuSO_4$ treatment, the experimental fish exhibited increased excretory activity as a means of expelling toxins from their bodies. Prolonged exposure to $CuSO_4$ resulted in higher excretion levels. Sometimes, body released mucus as mechanism to neutralize the toxic effects from metals. After exposure, the fish produced more excretory waste, while a slight decrease in excretion was observed in the BFE-treated groups.

Figure 4: Recovery response showed by the group of fishes after treated with BFE after exposure to the CuSO4.

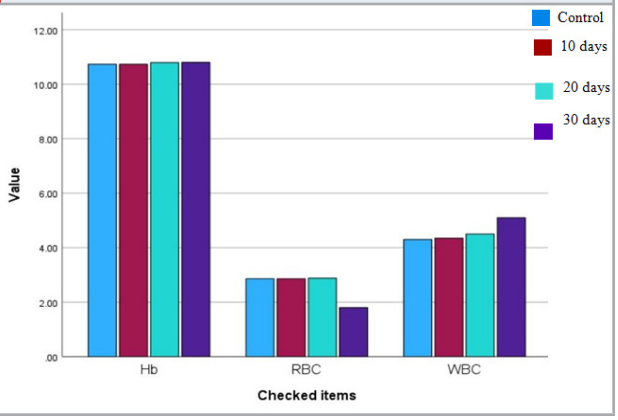
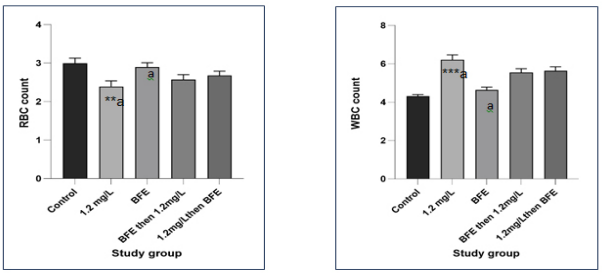


Figure 5: Effects of CuSO4 on haematological parameters of BFE pretreated Channa punctatus



Haematological analysis: The fish in each of the five groups—control, BFE treated, copper sulphate treated, BFE pretreated before being exposed to $CuSO_4$, and $CuSO_4$ treated before BFE treatment— had a differential count .The experimental data is documented in a table that suggests $CuSO_4$ interferes with the body's immunoreaction. Even while the fish treated with BFE and exposed to $CuSO_4$ do not exhibit total normalcy, the pretreatment group's blood parameters show a comparable range, which unquestionably demonstrates the antitoxic action of BFE.

The fish group that received only BFE treatment had blood characteristics that were similar to the control group, with a small increase in WBC count and higher levels of both haemoglobin and RBC counts. After Channa punctatus that had previously been exposed to chemicals received BFE, there was a modest increase in the number of red blood cells and haemoglobin, with the white blood cell counts coming closer to those of the control group (Table 1.2). The values

for RBC and hb count mentioned above showed a significant (P<0.05) decrease when compared to the control group while WBC count showed a significant increase (P<0.01) when compared to control group.

The reduction in haemoglobin content and low RBC count as compared to control appear to be a direct effect of copper sulphate toxicity which is reported to interfering haemoglobin synthesis by different toxicants like other heavy metals and pesticides.The revival effect on haemoglobin content erythrocyte count following BFE treatment have been clearly revealed in present findings which suggested the protective tole of antioxidants from BFE as an effective dietary factor in neutralizing toxic stress due to chemical pollutant exposure.In response to $CuSO_4$ treatment, the experimental fish exhibited increased excretory activity as a means of expelling toxins from their bodies.

Table 1.2 Haematological indices after pretreatment of BFE on *C. punctatus*

	Control	Days of exposure		
		10 days	20 days	30 days
Hb (g/dl)	10.73	10.73	10.79	10.8
RBC (106/µL)	2.86	2.86	2.88	2.89
WBC (103/µL)	4.3	4.35	4.5	4.6

Table 1.3 Effect of copper sulphate on haematological parameters of *C. punctatus*

	Control	Days of exposure		
		10 days	20 days	30 days
Hb (g/dL)	10.73	8.91	7.62	5.7
RBC (106/µL)	2.86	2.46	2.08	1.8
WBC (103/µL)	4.3	4.78	4.92	5.1

Table 1.3 Effect of copper sulphate on haematological parameters of *C. punctatus*

	Control	Days of exposure		
		10 days	20 days	30 days
Hb (g/dL)	10.73	7.6	8.5	9.2
RBC (106/µL)	2.86	1.88	2.39	2.62
WBC (103/µL)	4.3	4.78	4.52	4.4

Sometimes body releases mucus to neutralized the against the toxic from the heavy metals. Prolonged exposure to $CuSO_4$ resulted in higher excretion and releases more mucus levels. After exposure, the fish produced more excretory waste, while a slight decrease in excretion was observed in the BFE-treated groups.The revival effect on haemoglobin content erythrocyte count following BFE treatment have been clearly revealed in present findings which suggested the protective tole of antioxidants from BFE as an effective dietary factor in neutralizing toxic stress due to chemical pollutant exposure.

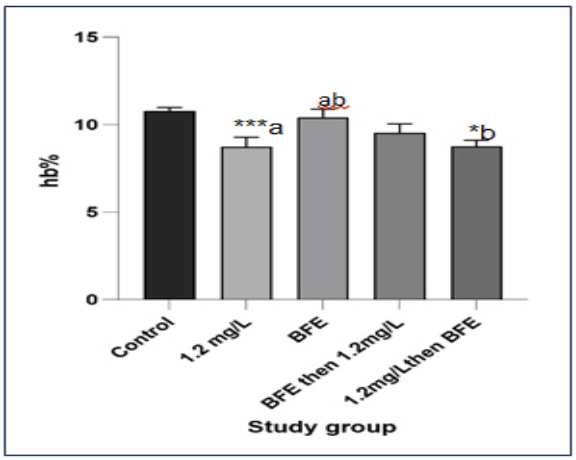
BFE on RBC count (B) Effect of $CuSO_4$, BFE, BFE then $CuSO_4$, $CuSO_4$ then BFE on WBC count (C) Effect of $CuSO_4$, BFE, BFE then $CuSO_4$, $CuSO_4$ then BFE on haemoglobin percentage. Value is significantly different from the control at $p < 0.001$ (***), $p < 0.01$ **), $p < 0.05$(*). (A) In RBC count, similar letters above the bar indicate values are significantly different at $p < 0.01$ (a), and (a). (B) In WBC count, a similar letter above the bar indicates values are significantly different at $p < 0.001$(a) and (a), and in hb estimation at $p < 0.001$ (a), (a) (b) and $p < 0.05$ (b).

Table 1.5 Effects of CuSO4 on haematological parameters of BFE pretreated *Channa punctatus*

	Control	Days of exposure		
		10 days	20 days	30 days
Hb (g/dL)	10.73	10.8	10.67	10.60
RBC (106/µL)	2.86	2.88	2.70	1.99
WBC (103/µL)	4.3	4.48	4.52	5.4

punctatus's blood parameters. (A) Effect of CuSO4, BFE, BFE then $CuSO_4$, $CuSO_4$ then BFE on RBC count (B) Effect of $CuSO_4$, BFE, BFE then $CuSO_4$, $CuSO_4$ then BFE on WBC count (C) Effect of $CuSO_4$, BFE, BFE then CuSO4, $CuSO_4$ then BFE on haemoglobin percentage. Value is significantly different from the control at $p < 0.001$ (***), $p < 0.01$ **), $p < 0.05$(*). (A) In RBC count, similar letters above the bar indicate

Figure 6: Histogram showing the effects of BFE against Copper- induced toxicity on C.

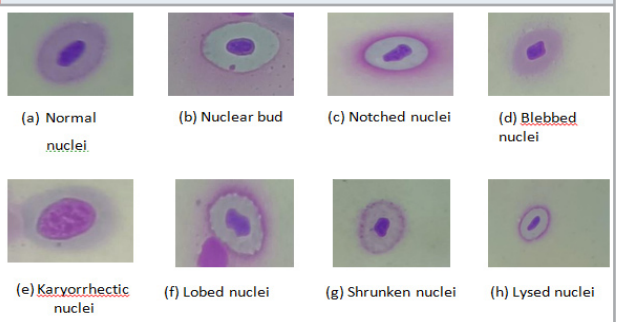


C

verage and SD (error bars) of the three specimens tested are displayed in each of the columns of the histogram (n = 3).The percentage of different types of leucocytes shows abnormalities The development of neutropenia

{high count of neutrophil),lymphopenia (ow levels of lymphocytes), eosinophilia (Increase no of eosinophil) and clumping of platelets occurs due to the toxic action of copper sulphate.

Figure 7: Micrograph showing nuclear abnormalities (NAs) in blood cells of C. punctatus in all the Groups at 100x magnification



Assessment of nuclear abnormalities: Abnormalities of nuclei (Nas) were observed in the peripheral blood cells, which were identified previously by several authors (Anbumanii and Mohankumar,2011; Shahjahan et al., 2020; Sarangi, 2021). In comparison to the control group (Fig. 1.7a), various nuclear irregularities were observed. These included (Fig. 1.7b) nuclear buds(NB), characterized by micronucleus-like structures attached to the nucleus.

Additionally, (Fig. 1.7c) nuclei displaying vacuoles and significant invagination lacking nuclear material, were designated as notched nuclei (NN). (Fig. 1.7d) Blebbed nuclei (BN) exhibited minor invaginations in the nuclear membrane. (Fig.1.7e) Karyorrhectic nuclei (KN) were marked by fragmented and disrupted nuclear structures. (Fig. 1.7 f) Lobed nuclei (LN) presented with two or more differentiated lobes. (fig.1.7 g) Shrunken nuclei (SN) indicated reduced nucleus size due to hypoxic conditions. Lastly, (Fig. 1.7h) lysed nuclei (LN) displayed incomplete or ruptured nuclear integrity. These observations contribute collectively to a deeper understanding of nuclear anomalies and their implications in *C. punctatus*. In the present work high percentage of shrunken nuclei and lysed nuclei and few blebbed nuclei, nuclear bud, and lobe nuclei in individuals exposed to copper sulphate also suggested that the metal can act as toxicants that affects nuclear membranes of ythrocytes. The erythrocyte from toxic changes were found closer to control after BFE treatment.

Histological analysis of liver: After a 30-day exposure, there was increased in necrosis as dose of chemical increased the higher the concentration of $CuSO_4$ the higher the chance of necrosis found in the hepatocytes. Similarly, the damage percentage of vacuolization and pyknotic nuclei are observed, respectively, after the completion of the experimental period. Vacuolization (fig 1.8 c), pyknotic nuclei (fig 1.8d), necrosis (fig 1.8e), and cytoplasmic degeneration (fig 1.8 b) as well as sinusoids (fig 1.8 f) were noted in all the treated Groups with an escalating pattern and dose-dependently; however, the greatest modifications were seen in Group IV, which was given the highest dosage.

Zeng et al. (2020) and Zebral et al. (2019) suggested that fish may have developed mechanisms to cope with copper exposure, potentially reducing liver damage. In their study, continuous exposure to copper sulphate caused vacuolization, necrosis, and cytoplasmic degeneration in fish liver cells, resembling previous observations.In another group, after exposure to $CuSO_4$, BFE treatment started and studied. There were subtle modifications observed in the hepatic cord structure, mild adjustments in cell arrangement, and slight alterations in nuclear structure. These changes suggested a minimal deviation from the effects seen in chemical ($CuSO_4$) treatment. In similar analysis of $CuSO_4$ exposure to the BFE pretreated fishes, the damage were found lesser compare to $CuSO_4$ such as decreasing in vacuolation, didn't progress necrosis rapidly. These findings suggested the protective role of BFE against metal toxicity.

Figure 8: Microphotographs taken from control and exposed Groups of C. punctatus's liver segment. Liver section demonstrating (a) vacuolization, (b) Cytoplasmic degeneration, (c) Pyknotic nuclei, and (d)Necrosis induced by CuSO4.X100 observed at 100X

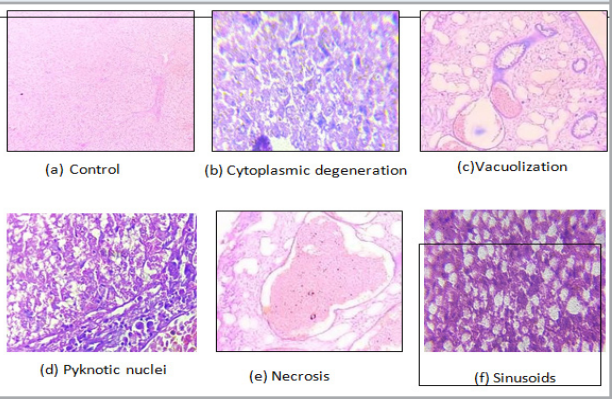
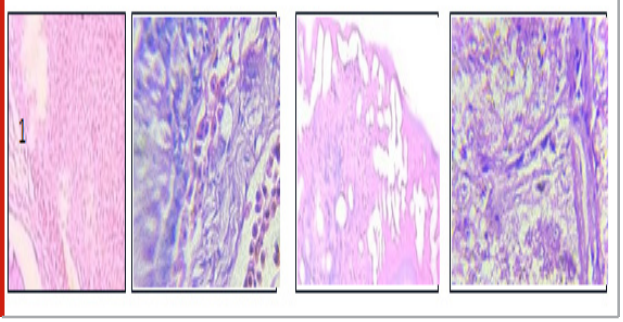


Figure 9: Hepatic tissues from different groups including 1, BFE; 2, CuSO4; 3, BFE then CuSO4; & CuSO4 then BFE. X 100

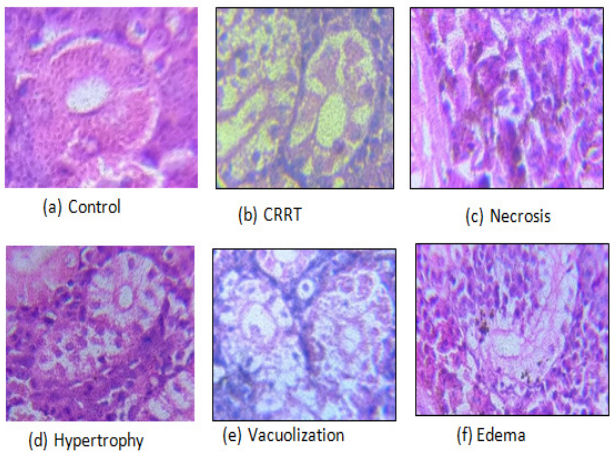


A regular mass of parenchyma cells makes up the liver of *C. punctatus* in its natural structural configuration. Hepatocytes, or polygonal cells with rounded nuclei, are seen in the histology of the liver and are grouped in the bile ducts, blood arteries, and liver parenchyma. The histopathological results of fish liver tissue from the 20-day exposed and control groups of *C. punctatus* are shown

in Figure 1.6. The most significant change that began in the liver was vacuolization, pyknotic nuclei (Fig. 1.6 d), necrosis (Fig. 1.6e), cytoplasm degeneration (Fig. 1.6 b), and Sinusoids (Fig. 1.6 f). Whil1e cytoplasmic degeneration exhibits the highest damage percentage of all types of damage, the liver tissue of the control group was in good condition and showed no signs of damage.

After a 30-day exposure, there was increased in necrosis as dose of chemical increased the higher the concentration of $CuSO_4$ the higher the chance of necrosis found in the hepatocytes. Similarly, the damage percentage of vacuolization and pyknotic nuclei are observed, respectively, after the completion of the experimental period. Vacuolization, pyknosis, necrosis, and cytoplasmic degeneration were noted in all the treated Groups with an escalating pattern and dose-dependently; however, the greatest modifications were seen in Group IV, which was given the highest dosage.

Figure 10: Micrographs of the kidney section taken from the exposed and control Groups of C. punctatus. Section of the kidney displaying the conditions: (a) control; (b) cavity reduction in the renal tubule (CRRT); (c) necrosis; (d) hypertrophy; and (e)vacuolization brought on by CuSO4 exposure shown at 100X



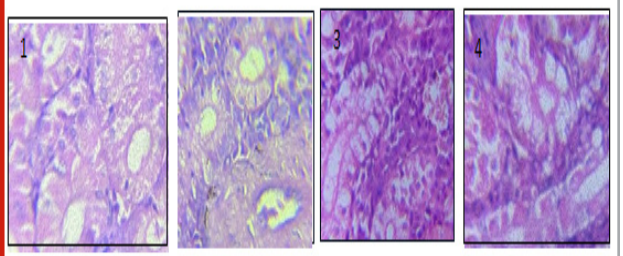
Histological analysis of kidney: In the untreated fish group, kidney sections exhibited a typical structure characterized by Bowman's capsules neatly arranged within renal tubules. The component of kidney tissue is altered by repeated exposure to $CuSO_4$, and the alterations in the test animal's kidney are expressed as a percentage of damage. Comparatively to the control group (Fig. 1.10a), the kidneys exposed to sublethal concentrations of $CuSO_4$ after 30 days showed multiple changes.

Cavity reduction in renal tubule (CRRT) (Fig. 1.10b) was the most significant alteration seen in the renal tubule, while necrosis (Fig. 1.10c), hypertrophy (Fig. 1.10d) and vacuolization (Fig. 1.10e) were the most frequent modifications in the tubules. Kidney tissue of Group 1 was healthy and did not show any damage. The CRRT damage were observed highest in the animals treated with highest dose, i.e., 1.2 mg/l of $CuSO_4$. However, necrosis, hypertrophy and vacuolization was observed in most part of the tissue. Among all four kinds of damage, the percentage of CRRT was the highest in the kidney tissue.

The animals grow increasingly lethargic and exhibit a noticeable inhibition of active movement as the exposure period lengthens. It was shown that feeding habits changed when mucus output and excretion increased. This could be because the metal, copper sulphate, gets absorbed by intestinal cells and interferes with the digestion process. The decrease of scale and discolouration around the lesion on their heads was another obvious alteration. Studying fish behaviour is highly valuable, as behavioural changes are linked to physiological biomarkers in aquatic species, reflecting their responses to biotic and abiotic environmental factors

Alterations in swimming patterns indicate fish avoidance or attraction in response to xenobiotic exposure. When exposed to foreign substances, gills utilize solitary chemosensory cells, taste, or pain perception to detect and react to the exposure. The connection between swimming patterns and chemical avoidance was demonstrated in Lake Whitefish (*Coregonus clupeaformis*) exposed to 0.2 µg/L of cadmium, where a dichotomous response was noted (Price 2013). Additionally, an upward swimming pattern towards the water surface is observed in response to oxygen depletion in copper-contaminated water.

Figure 11: Renal tissues from different groups including 1, BFE; 2, CuSO4; 3, BFE then CUSO4; & 4, BFE then CuSO4. (X 100)



The observation revealed that the metal had a general harmful effect on fish physiology, including changes in motility as well as histological damage to key organs like the liver and kidney. The haematological study's conclusions demonstrated a significant level of physiological stress since exposure to $CuSO_4$ significantly altered the blood picture. Blood is an extremely sensitive marker of both internal and external stress brought on by a variety of circumstances. The current results show that the number of red blood cells decreased in proportion to the number of days of exposure, whereas the number of white blood cells increased from the moment copper sulphate therapy began.

An increase in $CuSO_4$ dosage was likewise associated with a decrease in haemoglobin %.

Blood parameters are considered as pathophysiological indicators of the whole body and lawful are important in diagnosing the structural and functional study of fish exposed to toxicants (Adhikari et al. 2004). When assessing the combined impact of zinc and lead on the haematological parameters of carp, a synergistic effect was observed on red blood cell count, leukocyte count, and haemoglobin concentration. Comparable alterations were noted in *Channa punctatus* upon exposure to copper sulphate.

This study on haematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anaemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Decreased availability of oxygen generally causes increased synthesis of haemoglobin, release of blood cells from storage sites, and enhanced erythropoiesis.

Adakole (2012) demonstrated that exposure of *C. gariepinus* to effluents from metal finishing companies initially increased red blood cell (RBC) count, but this count decreased after prolonged exposure. In the current study, the decrease in total RBC count along with reduced haemoglobin content could be attributed to the detrimental effects of pollutants on erythrocytes, which were observed after chronic exposure. This could potentially impact the viability of the cells, a phenomenon also noted by Karuppasamy (2000). In total WBC, increase in total WBC count in the present study is the result of direct stimulation for the defense from decrease due to the preserve of heavy metals. Progressive increased levels of total WBC count have also been repeated.

Chandanshive et al. (2012) also reported decrease in RBC of fish Labeo rohita after exposure to mixture of heavy metals. All these reports are in agreement with the present study of reduction in total RBC count and Hb content of fish from polluted sewage fed pond due to the inhibition of aerobic glycolysis curtailing of iron and haemoglobin via the lowered energy status in fish. Leucocytes is directly proportional to severely to stress condition in maturing fish and as a result of direct stimulation of immunological defense due to the presence of heavy metal.

Hence results of the present investigation have confirmed that stress due to heavy metals present in water create haematological disturbances erythrocyte destruction (haemolysis) and leucocytes in fish population affecting immune system and making the fish vulnerable to diseases. Therefore, the fish is provided as a bioindicator of deteriorating water quality and due should be taken to monitor the environment.In fish, exposure to chemical pollutants can induce either increase or decrease in haematological indices such as haemoglobin (Hb), red blood cells (RBCs) & so on are used to assess the functional status of oxygen carrying capacity of blood stream and used as an indicator of pollution in aquatic environment (Shah & Altindag, 2004).

The hepatocytes show heterogenicity in size and were found to be undissociated in the present investigation. Cell damage were evident at places lesions of various size were present. Hepatocytes of large size were found to form disorganized running in all direction. BFE pretreatment for 30 days followed by copper sulphate revealed that many of these structural defects do not exist and the cell surface and other cellular features were more less similar to the normal hepatocytes.The haematological and biochemical profiles of fish *Labeo rohita* were examined using the medicinal plant *Curcuma amada* (Malik et al. 2019). The plant extract was combined with salt and fish feed powder, and after 30 days, the fish had much more red blood cells, white blood cells, serum protein, and globulin. The herbal diet also preserved liver problem enzymes, indicating enhanced health.

Kaur et al. (2018) conducted a study investigating the impact of copper on the liver histology of *Labeo rohita*. It was discovered that there was a positive correlation between the concentration of copper exposure and the severity of liver damage. This damage included vacuolation, congestion, and necrosis. Similarly, the study by Noureen et al. (2018) on the liver histology of *Cyprinus carpio* (common carp) exposed to copper showed severe damage, including degeneration, necrosis, and fibrosis. Their findings revealed that exposure to higher concentrations of copper resulted in dose- dependent liver damage in the fish. In contrast, the research done by Sangeetha and Aruljothi (2019) on the liver histology of common carp *C. carpio* exposed to copper showed mild damage, including vacuolation and lipid accumulation.

The kidney plays a crucial role in excretion, osmoregulation, and maintaining overall bodily equilibrium. Additionally, it facilitates selective reabsorption, aiding in the regulation of blood and body fluid volume and pH balance, as well as erythropoiesis.

In a study conducted by Wu et al. (2019) investigated the impact of copper treatment on the kidney histology of *Gobiocypris rarus*. They found moderate damage, including tubular necrosis, interstitial edema, and cellular infiltration. The authors suggested that copper accumulation in the kidney could impair renal function and potentially lead to fish mortality. Abdel et al. (2021) reported kidney damage in *Oreochromis niloticu*s exposed to copper. The kidney showed severe tubular necrosis and interstitial fibrosis, leading to decreased renal function. The study by Tavares-Dias (2021) on the kidney histology of fish *Labeo rohita* exposed to copper showed congestion of blood vessels, tubular necrosis of glomerulus, interstitial oedema, and cellular infiltration.

According to the study's findings, *C. punctatus's* copper buildup caused nuclear anomalies, histological alterations in fish organs like the liver and kidneys, a decline in RBC and Hb, rise in WBC, and other effects. Under the experimental parameters mentioned above, the BFE therapy demonstrated a recovery and a neutralizing effect in response to the toxic effects of copper sulphate, encompassing both haematological and histopathological criteria. The most

significant impact was seen in the recovery of blood parameters (RBC, Hb, and WBC).

As there is currently no treatment for copper intoxication and because the metal's abundance in the environment poses a threat to future generations as well as fish populations in aquatic bodies, it is strongly suggested by the current experimental findings that using high concentrations of banana flower as a raw ingredient in diet preparation may enhance the body's immunocompetence and serve as a useful tool in the fight against copper intoxication. Thus, it is evident from the results of the current study that antioxidants have a special role in preventing tissue damage as well as in reviving and shielding pathological damage caused by $CuSO_4$. It is anticipated that these results would pave the way for the use of banana inflorescence as a phytochemical instrument to mitigate the harmful effects of heavy metals on humans and other animals.

CONCLUSION

Adult *C. punctatus* has been shown to exhibit early behavioural and morphological changes in response to copper contamination in the aquatic environment, even at low concentrations. Prolonged exposure to copper sulphate was found to cause morphological and behavioural alterations. In addition to exhibiting vacuolation, necrosis, and hypertrophy, cytoplasmic degeneration were also observed in the tissues. The study concluded that the accumulation of copper in *C. punctatus* resulted in the reduction of RBC and Hb, and increase of WBC as well as nuclear abnormalities, and histological changes in organs such as the liver, and kidneys of the fish.

In the above experimental parameters, the BFE treatment exhibited a revival and neutralizing action in response to copper sulphate induced toxic impact including haematological and histopathological parameters. The most important effect of antioxidants was observed in recovery of blood parameters (RBC, Hb, and WBC). This research underscores the potential adverse effects of copper accumulation on fish health, emphasizing the necessity for effective measures to prevent such accumulation in aquatic environments. These findings can inform the development of strategies for the sustainable management of aquatic resources and ensure the long-term well-being of fish populations. To further understand the underlying mechanisms of copper- induced toxicity and create viable mitigation methods, more research is necessary.

Ethical Clearance: All ethical clearances have been obtained vide The Institutional Ethical Committee of the Institute, Department of Zoology, The Assam Royal Global University Guwhati, Assam India.

Author declares no Conflict of interest:

Data Availability: Data are available with the corresponding author.

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An Updated Review on the Role of Certain Physicochemical Parameters in Regulating Fish Diversity of Tropical Aquatic Ecosystems

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ABSTRACT

Numerous physicochemical factors found in aquatic environments have far reaching impact on fish growth and diversity. Contamination of aquatic resources has become a global environmental problem that threatens both the aquatic environment and the fishes, consequently affecting human health. Environmental stressors such as pollution, habitat loss and degradation, and climate change can also have significant negative impacts on aquatic ecosystems and can reduce the overall health and biodiversity of aquatic environments and fishes living in such environments. The present review emphasizes how crucial physicochemical factors such as temperature, pH, dissolved oxygen, nutrition levels, flow patterns, and habitat connectivity can shape fish assemblages. Predicting fish population dynamics, managing fish communities, and preserving biodiversity in the face of environmental change all depends on the better understanding of these interactions. This review also aims to give a comprehensive grasp of how these aspects affect fish diversity by synthesizing findings from other research projects. Although the physico-chemical properties of water play such a crucial role in determining the life history as well as the distribution range of fishes and their diversity, there is a lack of information regarding the suitable range of water quality parameters in which freshwater fishes can persist, as well as the role of the physico-chemical parameters affecting their assemblages. The observed adverse impacts of these pollutants along with external factors on aquatic ecosystems and fish growth and diversity emphasize the future needs for effective pollution control measures, proper waste management, and sustainable practices to protect and preserve the already deteriorating water quality and consequent decline in fish populations.

KEY WORDS: Fish Diversity, Habitat Connectivity, Physicochemical, Water Quality.,

INTRODUCTION

In aquatic habitats, fish variety plays a crucial role in biodiversity, impacting ecological balance and the overall well-being of these settings. Fish species composition and abundance are mostly determined by physicochemical factors, which also include water temperature, pH, dissolved oxygen, nutrition levels, flow regimes, and habitat connectivity. Environmental stressors such as pollution, habitat loss and degradation, and climate change can also have significant negative impacts on aquatic ecosystems and can reduce the overall health and biodiversity of aquatic environments and fishes living in such environments (Weinke & Biddanda, 2018).

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Contamination and pollution of aquatic resources has become a global environmental problem that threatens both the aquatic environment, fishes and human health. The pollutants specially heavy metals and pesticides can cause severe destruction of aquatic ecosystem and induce risks for human health through consumption of contaminated fish. The presence of pollutants in aquatic habitats can vary in concentration and allotment depending on the specific location, sources of pollution, and environmental conditions (Gavrilescu et al., 2015).

The adverse impacts of these pollutants on aquatic ecosystems and human health emphasize the need for effective pollution control measures, proper waste management, and sustainable practices to protect and preserve water quality (Perkumien˙e et al., 2023). Some of the specified pollutants that have been extensively studied and documented include petroleum hydrocarbons (Asif et al., 2022).

According to Kasperson et al, (2022) reported that there are urgent demands for comprehensive methodological

approaches to evaluate the actual state of these ecosystems and to monitor their rate of changes. Physical and chemical measurements commonly form the basis of monitoring, because they provide complete spectrum of information for proper water management.

This review research aims to give a comprehensive grasp of how these aspects affect fish diversity by synthesizing findings from other research projects. The research covers a variety of geographical areas and aquatic system types, providing insights into both regional variances and worldwide trends.

Although the physico-chemical properties of water play such a crucial role in determining the life history as well as the distribution range of fishes and their diversity, there is a lack of information regarding the suitable range of water quality parameters in which freshwater fishes can persist, as well as the role of the physico-chemical parameters affecting their assemblages.

The aim of this review is to analyse the role of some water parameters of inhabitant water bodies and corelate if there persists any correlation between physico-chemical parameters and fish assemblage with regard to their diversity.

As the physico-chemical parameters of aquatic bodies primarily regulate the biology and physiology of inhabitant fish species, the diversity of fishes are directly affected by mortality, growth patterns, and other several important factors related to physico-chemical aspects of aquatic system, (Kisku et al., 2017). The water quality parameters can be roughly divided into three categories, namely (1) physical (e.g., temperature and pH), (2) chemical (e.g., dissolved oxygen, total hardness, and nutrients), and (3) biological (e.g., microbes). Earlier studies on fish showed species-specific tolerance ranges of these factors, like thermal tolerance (Anttila et al., 2013; Chretien & Chapman, 2016), dissolved oxygen tolerance (Franklin, 2014), pH tolerance (Oliveira et al., 2008), nitrogen tolerance (Bowser et al.,1983; Williams & Eddy, 1986; and phosphorous tolerance (Nordvarg, 2001).Elevated freshwater pH occurs primarily due to acidified rain or snow depositions having long-lasting effects on freshwater pH. Aquatic ecosystems may also be affected by climate change-related acidification due to the increased uptake of carbon dioxide from the atmosphere, humic acid (from the degrading organic matter) can be another cause of freshwater acidification (Steinberg, 2003 and Kroupova et al., 2005).

Recently the excessively introduced nutrients from sewage discharges, agricultural wastewater, and diffuse runoff could build up the sediment. Nitrogen and phosphorus concentrations in the sediments are influenced by several hydro-chemical and hydrodynamic conditions in the water column above the sediment. Processes leading to their release to the water column from underlying sediments are numerous. The environmental variables which appear to regulate the release rate from the sediments are temperature, dissolved oxygen concentration, pH value, and redox potential (Hou et al., 2013).

The temperature of the water and its changing patterns have significant impacts on biological communities' composition as it determines the metabolic demand of individual organisms (Brown et al., 2004). The change in water temperature can be linked with thermal discharges, land-use changes, agricultural and irrigation return-flows, flow modifications, inter-basin water transfer, modification to riparian vegetation, and global warming (Roy, 2014). Warming increases metabolic rates more rapidly than ingestion rates leading to energetic inefficiency and predator starvation, affecting the higher trophic levels disproportionately. Thus, the indirect effects of warming through the food web sometimes can be greater than direct.

Physiological Effects. These impacts are stronger in freshwaters, with relatively discrete ecosystem boundaries which constrain the species potential to range shifts for tracking thermal optima.

As ectotherms, fish cannot regulate their body temperature, dependent on the external environment, so warming may directly alter physiological functions like thermal tolerance, growth, metabolism, food ingestion, and reproduction. If increases in metabolic demand are not meet by increasing food availability or strategies to maximize energy intake, populations are likely to decline or go extinct (O Gorman et al., 2016). Temperature alteration has a profound impact on the dissolved oxygen contents in water. The dissolved oxygen (DO) contents ofwater decrease with increasing temperature. Fish growth, feed utilization, and the bodys innate immunity are adversely affected by low dissolved oxygen (Abdel-Tawwab et al., 2015).

Acidification causes fundamental changes to biological and ecological processes in the aquatic ecosystem. One of the main consequences is the disruption of the chemosensory abilities of aquatic organisms. Detection of chemical cues supports a wide range of decision-making processes to exhibit their social behaviour. A low pH has been shown to interfere with predator avoidance and the detection of foraging cues (Kleinhappel et al., 2019).

Fish diversity is primarily influenced by two basic physicochemical factors: pH and water temperature. In a blackwater stream in the Southeast United States, Miranda and Hodges (2000) discovered a strong relationship between pH, temperature, and fish assemblage structure. Similar to this, Wang et al. (2019) showed that the pH levels in Erhai Lake, China, changed the biomass and distribution of aquatic vegetation, which in turn had an indirect effect on fish habitats. The role of physico-chemical parameters in fish culture and growth is a crucial aspect of aquaculture. Physico-chemical parameters such as water temperature, pH, dissolved oxygen, and turbidity significantly impact fish growth, survival, and their overall health.

Water temperature is one of the most critical physico-chemical parameters affecting fish growth and metabolism. Optimal temperature ranges vary among fish species, but most fish grow best in temperatures between 20-30°C. pH is another essential parameter, as it affects the availability

of nutrients and the toxicity of certain substances. Most fish species prefer a pH range of 6.5 to 8.5. Similarly, dissolved oxygen is vital for fish respiration, and its levels can significantly affect mortality and growth of fishes. Nutrient concentrations and dissolved oxygen are essential for fish diversity and survival.

According to research conducted in the Nanak Sagar reservoir in Uttarakhand by Banothu et al. (2018), high DO levels support a wider range of fish species. Their research revealed a positive correlation between total alkalinity, pH, and DO and fish productivity. Levels of nutrients, especially phosphorus and nitrogen, are also very important. Diverse fish communities were sustained by nutrient-rich habitats, as observed by Miranda and Hodges (2000).

An earlier important observation was of Edward and Ugwumba (2010) in which they reported that there is an increase in the number of zooplankton including fish fauna during the rainy season that was linked to the influx of nutrient. All the fish fauna and phytoplankton indicated high productivity in the rainy season and decrease to dry season.

This could be the reason why some studies have reported that the fish fauna has a positive correlation to most physicochemical parameters with only negative correlation to total dissolved solids, conductivity, calcium, pH and alkalinity. The high levels of parameters like nitrates observed, usually build up during dry seasons and that are only observed during early rainy seasons. This also played a vital role in the abundance of both fish and phytoplankton. Fishes appear to be sensitive indicators of changes as they have a temperature tolerance range of 25°C to 30°C with less alkalinity of 16 to 20 ppt compared to ammonia in their natural waters. Clarias species have possessed an ability to tolerate adverse water quality and difficulty in aquaculture.

Lates niloticus are found to be less abundant in oxygen poor condition in water quality. The positive correlation of most fishes like Cladocerans with dissolved oxygen and biochemical oxygen demand can be an indication that the waters are unpolluted. Balogun et al. (2005) in Makwaye (Nigeria) made an observation that fish and phytoplankton in Mairua reservoir, indicated monthly variations in abundance which could be due to variations of physico-chemical parameters. Food condition is still considered an important factor affecting growth and reproduction of most species of fishes.

Fish variety is seriously threatened by climate change because it changes the physicochemical properties of water bodies. When comparing the macroinvertebrate populations found in streams in temperate and Mediterranean regions, Bonada et al. (2007) hypothesized that variations in water temperature and flow patterns will be exacerbated by climate change, which will have an impact on aquatic biodiversity.Technological developments like next-generation DNA sequencing have completely changed how fish diversity and ecological health are measured. The potential of environmental DNA (eDNA) for biomonitoring was emphasized by Baird and Hajibabaei (2012), opening up new avenues for tracking how environmental change affects fish populations.

Hughes et al (1987) have reported that fish quantity and distribution are impacted by aquatic ecoregions, stream typology, and environmental conditions, as this study examined. It was discovered that many ecoregions, each sustaining distinct fish communities, are shaped by regional environmental factors as climate and hydrology. Fish presence and abundance are also influenced by the stream typology, which includes substrate type and flow regime. These findings emphasize how important it is to manage and conserve fish populations while taking typological and regional variability into consideration.

In his research, Matthews (1988) offered a thorough analysis of the ecological traits of prairie streams in North America, emphasizing their importance as important study systems for ecology. The review explores how fish and macroinvertebrate communities in these distinct stream systems are affected by physicochemical parameters as temperature, nutrient levels, and flow regimes. By analyzing these patterns, the research offers valuable insights into the biological processes that shape North American prairie streams, improving our knowledge of their biodiversity and ecosystem functioning.

In his review Matthews (1988) also has presented the ecological traits of prairie streams in North America, emphasizing their importance as important study systems for ecology. The review explored how fish and macroinvertebrate communities in these distinct stream systems are affected by physicochemical parameters as temperature, nutrient levels, and flow regimes. By analyzing these patterns, the research offers valuable insights into the biological processes that shape North American prairie streams, improving our knowledge of their biodiversity and ecosystem functioning.

Using molecular data, the study of Ganasan and Hughes (1998) examined gender-biased dispersal in the goby species Pseudogobius olorum. The results showed that there was no discernible gender bias in the dispersal patterns, indicating that environmental factors like connectedness and habitat availability could have an impact on the dispersal behaviors. Predicting fish population dynamics and preserving genetic diversity depend on an understanding of these processes.Similarly, Miranda and Hodges (2000) investigated a blackwater stream in the Southeast of the United States, the influence of physicochemical parameters on fish assemblage structure was the main focus of this study, which highlighted the significance of these factors in controlling fish populations in stream ecosystems by finding strong relationships between fish diversity and environmental factors such temperature, pH, dissolved oxygen, and nutrient levels.

Later on Caissie (2006) examined the river thermal regimes and their effects on aquatic environments, emphasizing the ways in which temperature fluctuations affect fish distribution and variety. It adds significantly to the body

of knowledge on physicochemical parameters and fish variety by offering a thorough examination of the variables influencing river temperatures and the ecological effects they have. Following this Leprieur et al (2008) reported the influence of fish invasions on river systems around the world, with a focus on how human activity might impede natural processes. Understanding the factors that lead to fish invasions is essential to reducing their negative effects on fish diversity and ecosystem integrity. Invasive fish species outcompete native species and change the architecture of their habitats, which modifies the physicochemical conditions.

Boix, et al (2008) worked on the parameters governing macroinvertebrate assemblages in streams with a Mediterranean climate, where physicochemical properties like nutrient levels, conductivity, and water temperature were found to have had a significant impact on the food chain involving fish diversity. The effects of siltation and changed flow patterns on the plains minnow's ability to reproduce in a riverine environment were the main focus of the work of Gido et al (2011) . The disruption of spawning habitats and the impact on fish reproductive behaviors caused by altered flow patterns and increased sedimentation underscore the significance of preserving natural flow regimes in order to sustain fish diversity.

Baird and Hajibabaei (2012) investigated the potential of next-generation DNA sequencing for biomonitoring and evaluating ecosystem health was covered in this research. Researchers can learn more about fish variety and aquatic community composition by examining environmental DNA (eDNA), which opens up new possibilities for tracking how changes in the environment affect fish populations. The combined impacts of fish stocking, habitat modification, and land use on mercury levels in fish populations in northern Minnesota were examined in this study. The findings highlighted the important of accounting for a range of stressors when assessing the health of aquatic ecosystems by demonstrating how anthropogenic activities have a substantial impact on physicochemical features and cause mercury to bioaccumulate in fish tissues, (Kratzer et al 2014).

Banothu et al (2018) evaluated the ichthyofaunal variety and limnological features of the Nanak Sagar reservoir in Uttarakhand. From August 2016 to March 2017, thirty fish species were identified through monthly sampling. Fish output was shown to be positively connected with pH, specific conductivity, total alkalinity, dissolved oxygen, and clarity in the reservoir's water, suggesting that a variety of fish communities are supported by the water quality of the reservoir. The study of Rodrigues and Leunda (2018). looked at how fish assemblage structure in a transient Mediterranean river was affected by environmental factors. Fish species composition and abundance were highly influenced by variables such water temperature, flow velocity, and substrate composition, highlighting the significance of taking local environmental circumstances into account in dynamic aquatic systems.

Fish diversity and physicochemical properties are greatly impacted by human activities, such as pollution, altered habitats, and changes in land use. The intricate interplay between anthropogenic stressors and aquatic health were highlighted by Kratzer et al. (2014), who looked at the cumulative impacts of land use, habitat modification, and fish stocking on mercury levels in fish populations in northern Minnesota.The fish fauna diversity, particularly, composition and abundance varies with months and seasons, which may be due to fluctuation of physicochemical parameters and reduction in abundance of phytoplankton, which are the primary producers. Yaseen et al, (2022) reported factors such as light intensity; food availability, dissolved oxygen, and predation affect the population composition of zooplankton including fish fauna. Later on, Wang et al (2019) looked into how pH affected the biomass and distribution of aquatic plants in China's Erhai Lake. High nitrate levels (> 1mg/l) are not good for aquatic life as recently reported by Gharti and Liping, (2023).

These studies have demonstrated the intricate relationships between physicochemical factors and biotic components in aquatic ecosystems which can affect the composition and abundance of aquatic plants, abundance of food, which in turn influence the quality of fish habitat and biodiversity.

CONCLUSION

Maintaining fish diversity is crucial for thriving aquatic ecosystems and is influenced by physicochemical factors like temperature, dissolved oxygen, pH, and nutrient levels. Anthropogenic activities, such as pollution, habitat change, and invasive species, negatively impact these conditions. Effective management requires controlling invasive species, reducing pollution, and restoring habitats. Advanced tools like eDNA and remote sensing can enhance understanding and management. Integrating ecological, hydrological, and social approaches is vital. Collaborative efforts among scientists, policymakers, and stakeholders are essential for sustainable conservation of aquatic resources, benefiting both ecosystems and human communities.

This review provides a comprehensive grasp of how these aspects affect fish diversity by synthesizing findings from other research projects. The physico-chemical properties of water play such a crucial role in determining the life history as well as the distribution range of fishes and their diversity. The observed adverse impacts of these pollutants along with external factors on aquatic ecosystems and fish growth and diversity emphasize the future needs for effective pollution control measures, proper waste management, and sustainable practices to protect and preserve the already deteriorating water quality and consequent decline in fish populations.

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Prevalence of Antimicrobial Resistance Amongst Uropathogens from Coimbatore: A Single-Centre Study

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ABSTRACT

Antibiotic resistance is becoming a major threat and is increasing worldwide. The increasing prevalence of infections caused by antibiotic-resistant bacteria make the empirical treatment of Urinary Tract Infection (UTI) more difficult. The aim of the study is to find the antimicrobial susceptibility pattern of pathogens causing urinary tract infection and help to improve the effectiveness of empirical treatment. The pathogenic bacteria cultures were isolated from the patients with urinary tract infections in the district of Coimbatore, Tamil Nadu, India and its antimicrobial susceptibility pattern had been examined using ten different standard antibiotic discs. The antimicrobial activity was performed using Kirby Bauer disc diffusion method. The results had shown a considerable increase in the resistance pattern of the uropathogens. Around 80% of the pathogens were being highly resistant to most of the antibiotics studied. Among the antibiotics, Imipenem being most effective followed by Gentamycin. Ampicillin was found to be the least effective. Most of the pathogens are resistant to more than three different classes of antibiotics. Present data revealed a steady emergence of multidrug resistance among the uropathogens in this region. Frequent multi-centre studies are required to understand the development of antimicrobial resistance of the pathogens as well as to know the effectiveness of the antibiotics prescribed. Situation urges to develop better antimicrobial agents with minimal or no side effects.

KEY WORDS: Antibiotic Resistance, Antibiotics, Disc Diffusion Method, Urinary Tract Infections, Uropathogens,

INTRODUCTION

Microbial resistance to drugs has become a global public-health threat compromising the efficacy of antimicrobial chemotherapy. The magnitude of this problem has recently been acknowledged by the World Health Organization, which has launched a global strategy for the containment of antimicrobial resistance. The emergence and spread of resistance are universally acknowledged to be associated with heavy consumption of antimicrobial agents in clinical and veterinary practices, and the prudent use of antibiotics has been considered to be mandatory for the preservation of their therapeutic effectiveness for as long as possible. Within this perspective, a combination of misuse and overuse of antimicrobial agents, along with overcrowding and poor sanitation, is among the reasons given to explain the exceedingly high resistance rates observed in low-income countries, (Okeke et al 1999, WHO 2001, Bartoloni et al 2004, Gajdács and Urban 2019, Kulkarni et al 2019, Anthony 2024 and PACCARB Report 2024).

The increasing prevalence of antimicrobial-resistant organisms are an important public health problem and is of particular concern for hospitals and other health care settings, (Fridkin et al 2002) Patterns of resistance in health care facilities are not uniform; even institutions in the same city may have quite different observed patterns of resistance for a given organism, (Lubowski et al 2001). In the United States, urinary tract infections result in about 8 million health practitioner visits per year. A 2013 record from the Centers for Disease Control and Prevention (CDC) revealed that greater than 2 million people within the US alone become ill each year as a result of antibiotic-resistant infections,

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and 23,000 die from such infections (Whiteman, 2014, Anthony 2024).

Resistance to antimicrobial agents among clinically important pathogens within the community and hospital settings has compromised therapy and requires constant monitoring of emerging patterns, (Jones 2003). The discovery of potent and safe antimicrobial agents is arguably the single greatest health care advance in history. The availability of these agents will rapidly reduce the morbidity and mortality associated with a host of formerly fatal diseases, (Rice 2008, PACCARB 2024).

Urinary tract infections are the most common site of microbial infections in human beings; it is estimated that 150 million UTI'S occur worldwide, resulting in more than 6 billion dollars in direct health care costs, (Stamm and Norrby 2001). Studies from different parts of India have indicated that UTI during pregnancy leads to low birth weight babies, increased perinatal mortality, and premature births along with many acute and chronic sequelae in the mother (Roy et al 1974). Recurrent urinary tract infection (rUTI) is a serious clinical problem, yet effective therapeutic options are limited, especially against multidrug-resistant uro-pathogens (O'Brien et al 2015, Anthony 2024).

The unrelenting increase in the prevalence of antimicrobial resistance is of great concern. The urgency of the problem is compounded by the recognition that fewer new antimicrobial agents are introduced each year, (Lautenbach 2009). The present study is designed to study the antimicrobial sensitivity pattern of different pathogens causing urinary tract infections among the people in Coimbatore.

MATERIAL AND METHODS

Collection and maintenance of pathogens: 50 various isolates of urinary tract infection causing pathogenic bacteria were collected from Sri Ramakrishna Hospital, Coimbatore from July to September 2013 and maintained on nutrient agar slants in cold room at 4°C.

Culture Media: Muller-Hinton Agar was prepared according to the manufacturer's instruction, autoclaved, and dispensed at 20ml per plate in 12 X 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use, (Lakshmanan et al 2012).

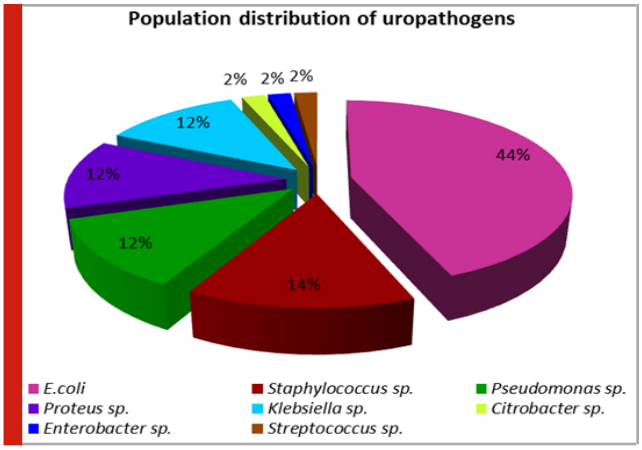
Antibacterial assay: Using the Agar disc diffusion method, Muller-Hinton agar plates were swabbed with the overnight grown culture of each microorganism. The antibiotic susceptibility discs were placed on each plate and incubated at 37°C for 24 hours (Lakshmanan 2013). The standard antibiotic discs used are Ampicillin (A), Cefpodoxime (CEP), Cefuroxime (CU), Cefepime (CPM), Piperacillin / Tazobactam (PT), Erythromycin (E), Gentamicin (G), Ciprofloxacin (CF), Co-Trimoxazole (CO) and Imipenem (I) were obtained from Hi-media.

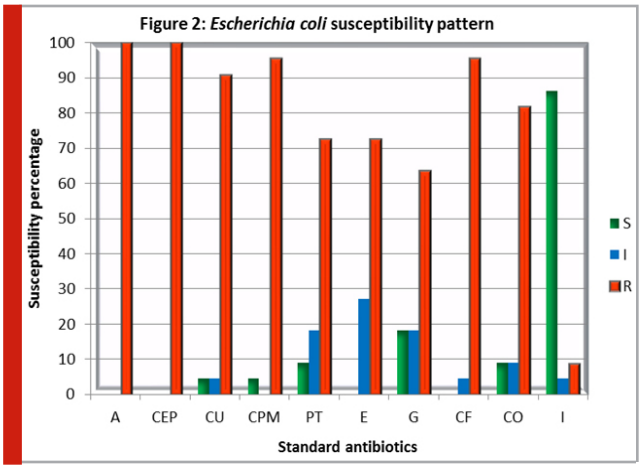
The Antibacterial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation (Krishnavignesh et al 2013). The susceptibility profiles of each pathogen against standard antibiotics were interpreted as per Clinical and Laboratories standard Institute (CLSI) guidelines.

Statistical Analysis: The results are expressed as the mean ± S.E.M. The results obtained from the present study were analyzed by using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

RESULTS AND DISCUSSION

The collected bacterial population is comprised of 8 different species which are *E. coli*, *Pseudomonas* sp., *Klebsiella* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Citrobacter* sp., *Enterobacter* sp. The population distribution displayed a clear dominance of E. coli over both the gram-positive and gram-negative uropathogens studied (Figure 1). The antimicrobial susceptibility pattern of each uropathogens was examined and their percentage was represented in charts (Figure. 1 – 8). In comparison with all the antibiotics investigated Imipenem is highly effective while Ampicillin being less effective.

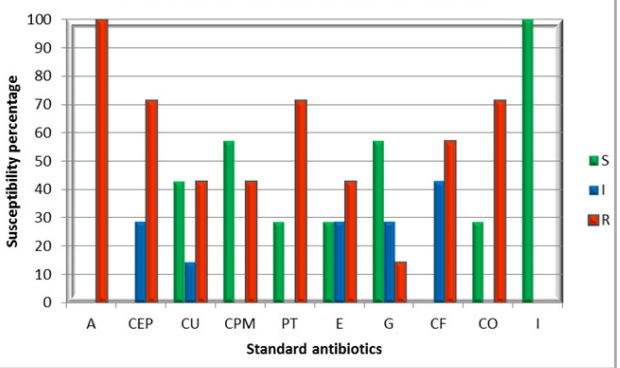




Along with urinary tract infections, the resistance level of uropathogens posing a huge threat to the medical well-being of humans across the globe. Urinary tract pathogens in Madagascar are becoming increasingly resistant to commonly used antibiotics that are readily available at a low price. This poses a real problem for the treatment

of community-acquired urinary tract infections (UTIs) in Madagascar (Randrianirina et al 2006).

Figure 3: *Staphylococcus* sp. susceptibility pattern



Foot note : S – Sensitive, I – Intermediate, R – Resistant, Ampicillin (A), Cefpodoxime (CEP), Cefuroxime (CU), Cefepime (CPM), Piperacillin / Tazobactam (PT), Erythromycin (E), Gentamicin (G), Ciprofloxacin (CF), Co-Trimoxazole (CO) and Imipenem (I).

Figure 4: *Pseudomonas* sp. susceptibility pattern

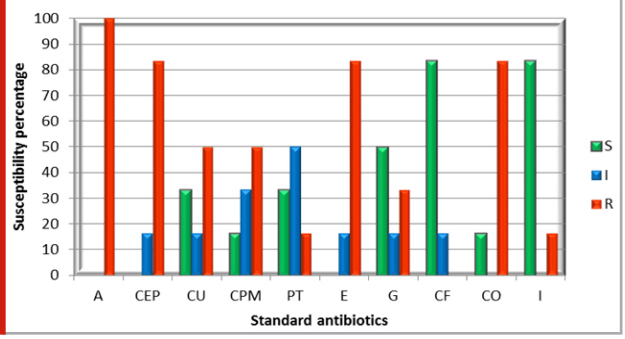
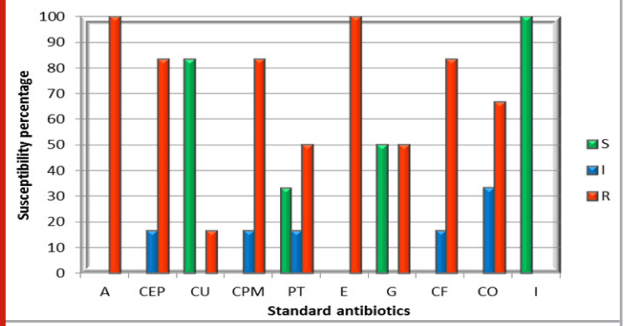


Figure 5: *Proteus* sp. susceptibility pattern



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Even India also experiences the same problem, Meta-analyses of the drug susceptibility results of various laboratories in India reveal an increasing trend in the development of resistance to commonly used antimicrobial agents in pathogens like *Salmonella, Shigella, Vibrio cholerae, Staphylococcus aureus, Neisseria gonorrhoeae, N. meningitidis, Klebsiella, Mycobacterium tuberculosis*, HIV, Plasmodium and others. One of the major concern is that there are no national data based on antimicrobial resistance in different pathogens yet known that the infectious disease burden in India is among the highest in the world, (WHO 2010, Kumar et al 2013).

Figure 6: *Klebsiella* sp. susceptibility pattern

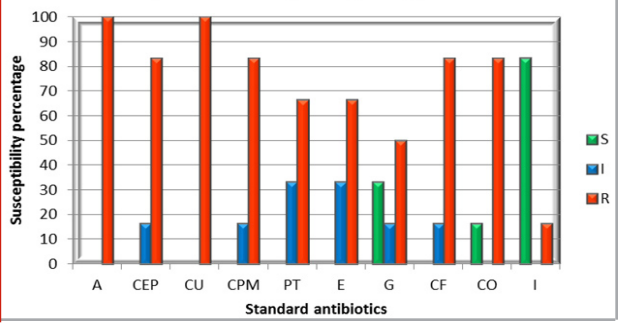
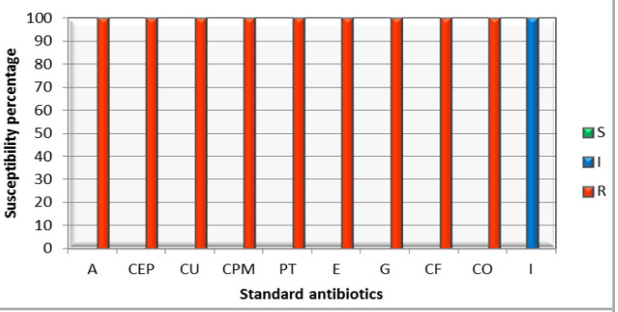


Figure 7: *Citrobacter* sp. susceptibility pattern



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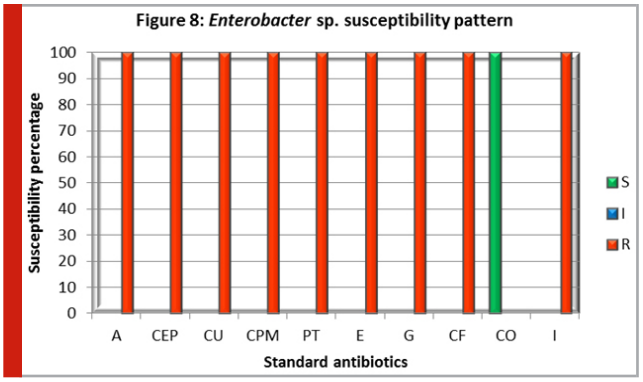
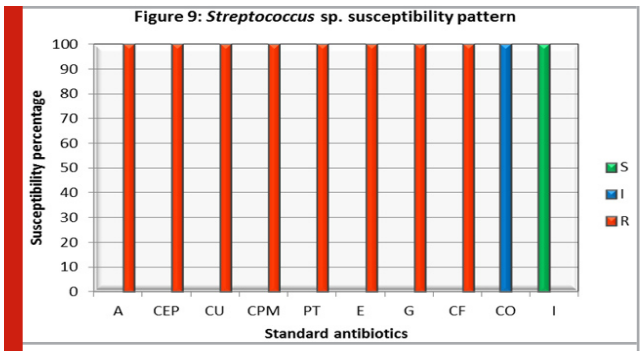
In the present investigation, the population of uropathogens was comprised of *Escherichia coli* (44%) followed by *Staphylococcus sp*., (14%), *Pseudomonas sp*., (12%), Proteus species, (12%), Klebsiella species (12%), and 2% of *Citrobacter* species, *Enterobacter* species and *Streptococcus sp.* (Figure 1). It has shown that *E. coli* was to be the major causative of urinary tract infections. The same pattern of distribution of uropathogens was reported in another 5-year study by Gupta et al (1999) which showed the presence of *Escherichia coli* (86%) followed by *Staphylococcus saprophyticus* (4%), *Proteus* species (3%), *Klebsiella* species (3%), *Enterobacter* species (1.4%), *Citrobacter* species (0.8%), and *Enterococcus* species (0.5%). Other, less frequent isolates in aggregate caused 1.3% of infections. It indicates that *E. coli* is being predominant amongst the other uropathogens. *E. coli* cultures shown a high level of resistance towards Ampicillin (A), Ciprofloxacin (CF), Erythromycin (E), and Cefpodoxime (CEP) while sensitive to Imipenem (I) followed by Gentamycin (G) (Figure. 1).

These results were supported by several other studies that stated the majority of *E. coli* isolates resistant to ampicillin, trimethoprim-sulfamethoxazole, or ciprofloxacin, (Zhannel et al 2000). The prevalence of fluoroquinolone-resistant

isolates of *E. coli* has been reported to be increasing over time in the United States and Canada. (Hooton 2003). 7.1% of urinary isolates of *E. coli* (2,763 of 38,835) were found resistant to three or more agents and considered multidrug-resistant. Among the multidrug-resistant isolates, 97.8% were resistant to ampicillin, 92.8% were resistant to trimethoprim-sulfamethoxazole, 86.6% were resistant to cephalothin, 38.8% were resistant to ciprofloxacin, and 7.7% were resistant to nitrofurantoin, (Karlowsky et al 2002, Sahm et al 2001).





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It shows that *E. coli* is gaining resistance to multiple antibiotics. The results of this study also raises the alarm of the emergence of multidrug resistance among the *E. coli* strains in Coimbatore. This may be due to the increase in strains harbouring an extended-spectrum β lactamase. *Staphylococcus sp.* cultures showed higher resistance towards Ampicillin (A), Piperacillin/Tazobactam (PT), Co-trimoxazole (CO), Cefpodoxime (CEP), while Imipenem (I) found to be highly effective with 100% inhibition followed by Gentamicin (G) and Cefepime (CPM). The study indicates the emergence of multidrug resistance among *Staphylococcus* cultures isolated from patients with urinary tract infections.

In support of the current findings already a high level of MRSA was reported in our country, which found 41% of methicillin resistance among 26310 isolates (India et al 2013). In India, Gram-positive infections, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence among invasive *S. aureus* isolates, have been increased exponentially from 29% in 2009 to 47% in 2014. Apart from MRSA, the rising prevalence of vancomycin-resistant enterococci (VRE), which ranges from 1 to 9% in India, has raised concerns, (Kulkarni et al 2019).

The majority of the *Pseudomonas* cultures investigated in the study were highly sensitive to Imipenem (I), and Ciprofloxacin (CF), followed by Gentamicin (G). While 50% of the isolates found intermediate to Piperacillin / Tazobactam (PT) and 33% were sensitive and found resistant to other antibiotics studied. This result was correlated with the study conducted in Pakistan, (Shah et al 2015) which reported 10% resistance towards Imipenam, 19.6% towards Piperacillin / Tazobactam (PT), 35% vs Gentamicin (G), and varies with the findings against 99% vs CU; 50% vs CF whereas we observed 50% and 100% resistance against CU and CF respectively.

This variation may be due to the indiscriminate use of antibiotics in the respective geographical areas. Besides the few contradictions, all these studies substantiate the development of multidrug resistance *in Pseudomonas* strains. Infection caused by this organism is difficult to treat because of the presence of its innate resistance to many antibiotics (β-lactam and carbapenem group of antibiotics), and its ability to acquire further resistance mechanisms to multiple classes of antibiotics, including Beta-lactams, aminoglycosides, and fluoroquinolones, (Pachori et al 2019).

Proteus isolates were shown higher sensitivity to Imipenem (I), Cefuroxime (CU) followed by 33% of sensitivity towards Piperacillin / Tazobactam (PT), and it has been found resistant to all other antibiotics investigated. The emergence of multidrug resistance observed among *Proteus sp*. in this study in Coimbatore district contradicts the findings of multicenter surveillance conducted in Taiwan that revealed decreased susceptibility of *P. mirabilis* to some broad-spectrum antibiotic particularly to the resistance rate against Imipenem. Besides the current study found zero percent resistance compared to 51.8%, however it correlates with the majority of the findings related to the increasing resistance level reported against different antibiotics including 3rd-generation cephalosporins and ciprofloxacin, in the past decade. Similar results were reported in Banglore, India found more than 80% of resistance in *Proteus sp*. This finding indicates the development of antibiotic resistance among *Proteus sp*. against multiple antibiotics. This might be due to a higher prevalence of ESBL- and/or AmpC β-lactamase- producers, (Wang et al, 2014, Navneeth et al 2002).

Klebsiella isolates showed good susceptibility to Imipenem (I) and moderately susceptible to Gentamicin (G) while it has shown resistance to all other antibiotics studied. A report in Uganda found concurrence with the current study showing 82% of *Klebsiella sp*. was resistant to at least three classes of antibiotics, (Stanley et al 2018).

In Tanzania, the resistance level of *Klebsiella* spp strains was cotrimoxazole (92.6%), ciprofloxacin (25.0%), and

gentamicin (38.2%) but our study in Coimbatore district displayed a considerably higher level of resistance of 83.3%, 83.3%, and 50% against the respective antibiotics. This might be due to the production of ESBL among the strains, (Moyo et al 2020).

All the above studies found Imipenem is being effective against *Klebsiella sp*. *Citrobacter* isolate found intermediate only to Imipenem (I) and exhibited resistance to all other antibiotics tested. The current study was found parallel with another which reported the majority of the urinary tract *Citrobacter* isolates were found to be resistant to cefotaxime, cephalexin, norfloxacin, ciprofloxacin, and the aminoglycosides. This may be because of the widespread use of broad-spectrum antibiotics, leading to selective survival advantage of pathogens, (Metri et al 2013).

Enterobacter isolate was susceptible to Co-trimoxazole (CO) while it was found resistant to all other antibiotics inspected. The most crucial finding was its resistance to Imipenem. *Streptococcus* isolate found sensitive to Imipenem (I) and intermediate to Co-trimoxazole (CO), to the rest of antibiotics, it displayed resistance. *Citrobacter*., *Enterobacter*, and *Streptococcus* isolates displayed a higher rate of resistance against different classes of antibiotics. A study in Hungary also reported the emergence and spread of ESBL-and/or carbapenemase-producing Enterobacter (Gajdács et al 2019).

Our study has shown Imipenem (I) as an effective antibiotic against the majority of the uropathogens investigated followed by Gentamycin (G). Though most of the organisms were not sensitive to Ciprofloxacin (CF) it was found intermediate in a considerable volume of the microbial population investigated. Piperacillin / Tazobactam (PT), Cefuroxime (CU), and Cefepime (CPM) had shown moderate activity. Co-Trimoxazole (CO), Erythromycin (E) and Cefpodoxime (CEP) were found to be least effective. Most of the reports worldwide found Imipenem to be one of the most effective antibiotics. Ampicillin resistance pattern varies among countries, was reported to be Ethiopia (17%), Italy (36%), UK (23%), USA (43%), Canada (33%), Europe (50%) and Norway (25%), (Bitiv et al 2017).

In contrast to the above findings our study in Coimbatore district, Tamil Nadu, India was found to be 100% against both the gram-negative as well as gram-positive uropathogens, these findings were supported by another study conducted at Banglore, India reported a 93% resistance among the gram-negative uropathogens, (Navneeth et al 2002). Studies from African countries and Iran also reported 100% and 89.29% resistance respectively against ampicillin, (GLASS Report 2017).

Though Imipenem was found to be an effective antibiotic amongst most of the organisms, still this study found a small fraction of microbial population resistant to it. It is an alarming signal since carbapenemase-producing pathogens are a significant clinical and public health concern that will drastically limit the therapeutic armamentarium, (Gajdacs and Urban 2019).

Several factors contribute to the emergence of antimicrobial resistance, they are indiscriminate use of antibiotics, intrinsic antibiotic resistance like efflux systems and producing antibiotic-inactivating enzymes, acquired antibiotic resistance like mutations, adaptive antibiotic resistance like biofilm mediated resistance, and microbial genomic plasticity. The constant changing of the microbial genome is a bigger threat than the infection itself. There are several mechanism microbes utilize to alter their genomes such as horizontal gene transfer, transposon-mediated genomic alterations and promiscuous nature of the gene and genomic products.

Some of the world's most common and potentially most dangerous infections are proving drug-resistant and most worrying of all is pathogens don't respect national borders. That's why WHO is encouraging all countries to set up good surveillance systems for detecting drug resistance that can provide data to this global system, (GLASS Report 2017 WHO 2018). This emphasizes the importance of the local resistance data which is crucial to any part of the world. Though the sample size of this study is small it provides the much needed local resistance data which reveals the first details on population distribution among the uropathogens and its antimicrobial resistance pattern towards the different class of antibiotics in the Coimbatore district, Tamil Nadu. With these initial insights, it warrants a detailed multicentric study on the surveillance of antimicrobial resistance patterns of uropathogens to design an effective protocol.

CONCLUSION

The present study revealed that the uropathogens are steadily gaining resistance towards most of the commonly used antibiotics. This is a clear warning signal over the emergence of multidrug resistance among the uropathogens in Coimbatore, India as well as all across the globe. Concurrent resistance to antimicrobials of different structural classes has arisen in a multitude of bacterial species and complicates the therapeutic management of urinary tract infections. Frequent prevalence studies on pathogens causing urinary tract infections are warranted to understand the ever-changing antimicrobial trends in their respective regions. Growing multidrug resistance limits the therapeutic options. This scenario urges the scientific and medical community to develop additional effective antibiotics in order to serve the community in a better way. With all this, we are in a situation to frame a strict operational policy of antimicrobial therapies and standard treatment guidelines.

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Scientific Research and Development in Indian Higher Education: Problems and Challenges

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ABSTRACT

India one of the fastest growing economies of the world, is riding on a wave of academic revolution, amidst its population almost reaching one and a half billion. Consequently, there is a fierce competition in good and useful education. With resources becoming scarce and costly, there are few choices left to the young generation in its quest for learning. And, we are at a time when science and technology is at crossroads, making decisions difficult for students to opt basic science or plunge into the state-of-the-art industry loving technology tools, giving quick revenues. In this communication, educational pitfalls, challenges, and opportunities are discussed in order to cope up with the advancement of science and technology. Unfortunately, one of the scariest problems is, the system of selection of highly qualified and deserving faculty in India's most of the colleges and universities. Of late, this has become a matter of great concern and redressal at the earliest. Those who once get selected in a dubious manner, remain nothing more than, academic parasites for rest of their career. Seldom, meritorious achievements, quality publications, research and development experience are taken into consideration. This has to be eliminated at the earliest so as to preserve the sanctity and high standards of our education system, set-up so painstakingly by our earlier generations.

KEY WORDS: Higher Education, Research, Development, Science and Technology ,

INTRODUCTION

Riding a wave of academic revolution, as a country of almost nearing one and a half billion, competition in Indian education is cutthroat. And, we are at a time when science and technology is at crossroads, making decisions difficult for students to opt basic science or plunge into the state-of-the-art industry loving technology tools, giving quick revenues. Be it some entrance exams, admission to a particular school or even getting into a particular college or a university – the children are pressed for time and they have to perform better than others in order to succeed; which ultimately proves to be detrimental not only to the health of the young generation, but is also a wrong approach towards the entire learning process.

Choosing science as a career for teaching and research in higher education is on a decline, as other fast-track job options with greater transparency are available in corporate areas. Recently, in the US a young brilliant academic, abruptly left her PhD to become a content provider on social media, earning millions.

The learning culture in the Indian education system is highly exam-oriented, academic success-driven as well as rote memorizing. On the one hand, where there are highly successful elements within the system such as Indian Institutes of Technology (IITs), and Indian Institutes of Management (IIMs), Indian Institute of Science and Research (IISERs), there is still an even larger gap in overall quality, dropout rates after primary education and ability to respond to the increasing skill intensity of the international labour markets demand.

Indias biggest educational disadvantages has been the lack of high quality government primary and secondary schools across the nation because, historically, out of all the sectors, in terms of funding, education has never been given much emphasis, the annual budgets speak of the tragedy, coupled with the fact that appointments in such institutions, followed by salaries and grants for developments, have always been questionable. Mainly for these reasons, the majority of the government schools lack basic infrastructure and amenities. This is more so prevalent in areas where most of the schools

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are inadequately endowed with even the most basic facilities such as ideal classrooms, sufficient washrooms, libraries, and laboratories. This poor infrastructure has an impact on the learning of the students and their development all around.

On the other hand, higher education has become expensive, most often denying the students from the low-income earner's family a chance to pursue their education. These paradoxes are the most problematic of the whole educational system, staring from primary to higher, ending in a chaotic situation for the public. Consequently, everyone is left with no option but to take a big loan to pay for the fees, or abandon the dream of getting an education. According to the findings of Skill India Report, (2019), and other reports from industry, special-purpose committees formed over the years have only served to reinforce the challenges and negative perception of the broader education sector, (The Indian Higher Education 2022).

In my career of more than four decades as a teacher and a researcher, I have seen teaching and research in our colleges and universities quite well. Appointments of faculty in colleges and universities have been the most crucial and challenging aspects of institutional development. Weak and unqualified candidates using all sorts of extra educational resources can be academic parasites for ever, damaging the very foundation of any institution.

Unfortunately, Indian system of selection of good and deserving faculty has mostly been questionable. There are very few instances of fair selection by large. Selection proceedings have often been challenged with little justice or almost none, as those who can manage the selection committees can also influence judgments using similar strategies. This is unheard-of in developed countries who give more importance to highly qualified and deserving candidates irrespective of cast and creed. What are the reasons for this disturbing and alarming situation which our temples of learning are facing of late ? Qualifications, experience, and other basic educational requirements have not changed much since the inception of institutions. Rather the calibre and potential of the candidates of the recent generations have risen but not with matching standards.

There is an over production of talent but with few takers of it. Political and social interference often dilutes the quality of education where ultimately the students and their future generations are at loss. Programs in engineering, computer science, and nursing consistently show the highest return of investments (ROIs), with lifetime premium earnings. For example, computer engineering graduates from top programs can expect lifetime earnings up many more times than other graduates. Business administration and economics degrees typically generate strong returns, financial managers with even a graduation for instance, accountants and auditors earn much more than pursuers of science have to toil for years, until they get their doctorates. Education is a big wheel in meeting the socio-economic, cultural and developmental needs of the nation and its citizens (Weber, 2011).

Indian institutions have started to improve their international rankings, research papers are rapidly increasing in international indexing databases such as Web of Science and Scopus. It can be observed that India has nearly tripled its count of Scopus-indexed documents in the ten-year period from 2010-2020 outgrowing every other country in the world. This increase seems unnatural compared to the more organic growth observed across the world in the major countries which have mature research ecosystems. Despite this quantitative growth in research papers published, none of the Indian universities rank among the best in the world in terms of research and development. After 2020, the rate of research publications has accelerated even further. For instance, till 2022 India had contributed over 550,000 documents in Scopus compared to a lowly 30,000 documents compared to second placed USA, (Gupta and Sawhney 2023).

One emergent trend in the higher education sector, is increased focus on quantitative data by the accreditation and ranking agencies. This has spurred the sector into a competitive overdrive with institutions vying for rankings and awards. This has also resulted in malpractices, systemic erosion, and gamification of the system with long term repercussions. Gupta and Sawhney (2023) have strongly argued against adopting purely quantitative measures for institutional assessment, suggesting measures which are holistic, broad-based, and better serve the needs of a country as diverse as India.

The last many years have seen the larger institutions enhance their fees significantly (FICCI Report 2021). The FICCI Report on the "Higher Education in India: Vision 2040" states that the cost of general and higher education has increased significantly over the last 10 years making it difficult for middle class Indians to afford it. An important trend of charging excessive fees from the students and burdening them with loans, which are difficult to repay, there has been a significant rise in the culture of hiring consultants, ranging from individuals, firms and companies which are mostly foreigners and are happier in revenues and converting the entire system consultant driven.

These experts take care of international faculty hiring and training, students' admission, facilities like arranging loans, housing. They also lure the institutes of international cooperation and signing the superfluous memorandum of understandings, serving as white elephants for the deserving. This almost leads to a vicious cycle of heavy investments, resulting in students suffering and not achieving their goals. Research shows that such an artificial and superficial system of faking excellence leads to short-term successes, inculcating a sense of artificial achievements, which lose meaning after a while. Other negative impacts of badly designed accredited systems include declining effects, cheating the system, task quality and privacy, (Thiebs et al 2014).

Gupta and Sawhney (2023) have concluded that quantitative measures, statistics and analytics are great tools for uncovering trends, as indicators of progress and determinants of productivity and outcomes. However, when they become

absolute measures of quality and strong incentives are linked to them, the system connives and collaborates to game them, which is not suitable for higher education.

In presence of an ever-rising stiff competition in older institutes of excellence like the IITs, IIMs and recent IISERs, most of the corporate like functionaries of private players in education, have been able to use quantitative outcomes to consolidate their brand value, admit a disproportionately large number of students and charge premium fees, without significant deliverables worth of their claims. This has impacted access to education for students who aspire to get admitted to such institutions and ultimately ruin themselves in the long run. Thus, it is time to review the present dispensation of ranking and assessing institutions based on purely quantitative outcomes.

Innovative qualitative assessments, selecting the deserving faculties, giving more importance to research and development, hands on trainings, use of sophisticated equipment, patents, good quality research publications of reputed publishers, doing away with forced PhD publications in predatory journals must be made mandatory. Selection of outstanding faculty is like creating and building a strong future for the institute. India needs to prioritize its own research by creating appropriate platforms for its publication and dissemination.

Around 2014 and onwards, Current Science, Indias premier fortnightly journal of Indian Academy of Science, Bangalore India published a series of communications on quality of research and education in Indian universities, including the doctoral theses which are literally manufactured on a large scale. Gunasekaran (2014) Ali (2014) pointed out the issues of publication ethics in the subcontinent, highlighting the prevailing menace and misuse of the fake impact factor-index concept, predatory journals, and the journal membership editorial board criteria. Evaluation of one's research quality in Indian universities and colleges for academic promotions has always been a complicated issue, often swayed by academic politics, nepotism and favouritism, (Ali 2014).

We have to make revolutionary and sweeping changes in our research set-up, including colleges and universities. The suggestions made by Gunasekaran 2014, Zare (2012, 2014) and Ali (2014), be strictly followed in all academic appointments, promotions and other research benefits of Indian institutions.

Stanford professor Richard Zare, appalled at the misuse of citation data and journal impact factors (IFs) in academic circles in countries like China and India, emphasized the critical importance of enlightened peer opinion in matters of granting tenure in research institutions. According to Zare (2012) Stanford and most other American universities do not pay much attention to the number of papers published, impact factors or H-Index of journals in which they are published. They depend entirely on the opinion of their tenured faculty members and outside experts. The evaluation of every faculty member is done annually, based on grants obtained, publications in top journals, and research projects completed.

Despite the concerns expressed by several others, (Keyser 2013, Pulverer 2013, Balaram 2013, Xue Li et al 2014 and Wouters 2014), on using journal IFs for assessing an individual's work, many funding agencies, assessment boards, research and development laboratories in India and other countries still use them as a surrogate measure of the quality of research by individuals for the selection and promotion of scientists and research fellows. In countries like China, South Korea and Turkey, scientists are paid cash incentives when they publish in high IF journals.

Similarly, Gunasekharan, (2014) has recommended that funding agencies will do well to look at the quality of research performed / reported instead of counting the number of papers. Otherwise, predatory journals will have a field day. Researchers also should explore the background of any journal by its publisher, place of publication, peer-review process, editorial board members and the quality of articles published in that journal before sending a paper for publication or accepting to be on its editorial board.

Ali (2014) had suggested that august bodies like University Grants Commission / and State Higher Education Commissions / Departments and other such boards must consider giving more credit to the quality of a research paper, rather than accepting at par papers published anywhere. At present, such fake research papers published in predatory journals make one a college or a university professor, frustrating those who strive hard for quality and publish with reputed publishers like Cambridge, Oxford, Pergamon, Springer, Taylor and Francis and others. Such a measure will naturally perish the predatory journals soon. An important aspect of assessing the quality of a paper would be to use the double-blind method by tenured expert faculty.

In spite of all the issues apparent in the Indian education system; however, it still looks good and has the potential to build a strong and robust educational system for the country. This is due to the fact that the spending is very less on our education as compared to others. India spends only 0.66% of its GDP on research compared to 2.44% by China, 2.74% by USA, 3.26% by Japan, 4.81% by Korea or 5.44% by Israel, (World Bank Data 2022). The world average for R&D spending during 2022 is 2.63% of GDP, which has gone up over the last 3 years, while Indian spending has remained stagnant.

Secondly, we have no dearth of talent, it is well known that Indian students have been the dominating force world-wide, our top 15 to 20 % students are truly world-class. We need to be patient with the process of "institution building" and realistic expectations should be set for institutions. Therefore, one can conclude that thanks to changes in the curriculum, or to the application of technologies rather than expecting the delivery of more money for the growth of credits helps to raise the quality of learning and the learning process becomes more significant.

The goal justified should concentrate on more effective strategies assuring learner individual development, equity, and the least cost. It will be a big relief to learn that if the right policies, innovations and the necessary commitment from all the stake-holders are in the right place, the future of education in India could be enhanced, matching to the glorious past, it had in the field of education and research.

CONCLUSION

Being one of the fastest growing economies of the world, India is riding on a wave of academic revolution, amidst its population almost reaching one and a half billion. Consequently, there is a fierce competition in good and useful education.With resources becoming scarce and costly, there are few choices left to the young generation in its quest for learning. And, we are at a time when science and technology is at crossroads, making decisions difficult for students to opt basic science or plunge into the state-of-the-art industry loving technology tools, giving quick revenues. It is recommended that despite all flaws and problems in higher education research and development in colleges and universities, much can be done to improve the dwindling standards of education, and a consequent decline in interest of students. Fair selection of highly qualified faculty, sans any kind of influence, developing basic science and technology infrastructure with the help of trained faculty must be on top priority. Once, the high education and research standards have been developed, the use of quality accreditation can make us achieve the much-required standards of education, which the country is known for.

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Alterations in Regulatory Matrix Related to Inflammation in Lungs of Calcium Carbide Exposed Mice

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ABSTRACT

Pulmonary disorders are established as consequence of air pollution. Emissions from vehicles, factories and combustion of biomass are major contributors to air pollution. Accumulation of pollutants in lungs develops several respiratory diseases like asthma, chronic obstructive pulmonary disease (COPD). Persistent inflammation due to long term exposure to toxicants causes permanent pulmonary tissue damage and often arises serious medical emergency. Calcium carbide (CaC_2) and its byproduct acetylene have been recognized as potential environmental toxicant. It is widely used in various commercial fields including fruit ripening and welding, cutting industries. In the present study we have investigated the inflammatory status and its consequences on lungs during inhalation of CaC_2 generated toxicants by mimicking similar situation of people who occupationally get exposed with the same. After 40 days of exposure by calcium carbide generated gas, real-time qPCR has been performed to investigate the level of Cox_2 and PGES mRNA. Apoptosis and T_{H17} as well as T_{reg} percentage have been estimated flow cytometrically. Levels of Cox_2 and PGES have been found to increase with the increase in CaC_2 exposure. Apoptotic cell percentage has been declined with increasing dose of toxicant. T_{H17} and T_{reg} cell percentage reduced as the dose of toxicant increased, indicating a balance between the two at 7gm CaC_2 exposure. Balance between inflammatory and anti-inflammatory condition of lung tissue has been attempted after chronic CaC_2 exposure, but it has been disrupted at the highest level likely due to accumulation of damaged cells as reflected by the spike of Cox_2 mRNA level in 7gm CaC_2 exposure.

KEY WORDS: CALCIUM CARBIDE, LUNGS, TOXICANT, INFLAMMATION, APOPTOSIS,

INTRODUCTION

Considering today's environmental situation, the quality of air we breathe every day is coming up as the predominant cause of several health issues. Especially, in industrial areas and large metropolitan cities the ambient air, mainly the emissions from vehicles and factories act as major conduits for airborne toxicant exposure (Kunovac et al., 2020; Lee et al., 2021). The fuels used in cooking, burning of wood and other materials used in fireplaces act as source of indoor air pollutants (Lee et al., 2021). Combustion of biomass fuel by traffic worldwide as well as domestic fire have been recognized as significant contributors to air pollution in developing countries (Laumbach and Kipen, 2012; Lee et al., 2021; Jiang et al., 2024).

The accumulation of pollutants in the lungs is responsible for triggering the airway inflammation, fibrosis and pulmonary dysfunction. These ultimately lead to exacerbation of the symptoms of chronic respiratory diseases like asthma and chronic obstructive pulmonary disease (COPD) (Lee et al., 2021).

Inflammation is a natural physiological process by which body responds to any injury or infection (Medzhitov, 2010; Chen et al., 2018). Though inflammation is a part of natural healing process, but long term exposure to toxicants causes

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persistent inflammation which may lead to permanent damage of lung tissues and alveolar structure (Wong, Magun and Wood, 2016). Severe lung inflammation may cause hypoxemia or hypoxia giving rise to symptoms like bradycardia, restlessness, cyanosis, dizziness etc. Chronic inflammation over long time may result in a progressive condition called bronchiectasis which ultimately may lead to hyercapnia causing medical emergency (Sarkar, Niranjan and Banyal, 2017; Hill et al., 2019; Defnet, Hasday and Shapiro, 2020).

Although calcium carbide (CaC_2) and its byproduct acetylene have been recognized as potential environmental toxicant according to related reports, it is widely used in various commercial fields. Calcium carbide decomposes in water forming highly flammable acetylene gas, a reaction discovered by Friedrich Wöhler in 1862. This property of calcium carbide is utilised in fruit ripening industry to hasten the fruit ripening process (Asif, 2012; Bini, Rajesh and Babu, 2022; Okeke et al., 2022). These chemically forced ripening not only minimizes the nutritive values of fruits but also makes the fruits toxic and increases the risk of health complications (Andrew et al., 2018; Cissé et al., 2020; Okeke et al., 2022).

Apart from the immediate symptoms like stomach discomfort, dizziness and vomiting, prolonged hypoxia may also affect the neurological system on consumption of these chemically ripened fruits in large quantities (Per et al., 2007; Okeke et al., 2022). Moreover, this toxicant gain access to body physiology through inhalation of left over powders, aerosols, and gaseous products of CaC_2 residues present on the fruits. Besides, this toxicant is also used by some food vendors to boil eggs and hard-to-cook beans (Okeke et al., 2022).

Apart from using in fruit ripening and food processing, calcium carbide is also utilised for the production of nitrolime (a fertilizer), desulphurization of iron in the process of steel making (Güthner and Mertschenk, 2000; Okeke et al., 2022). Calcium carbide generated acetylene gas is used as a raw material in chemical industry, especially for PVC production, also in carbide lamps as well as welding and cutting industries (Tajudeen, Akanfe and Adebayo, 2019; Okeke et al., 2022).

The workers in these industries are the most susceptible to the hazards caused by the toxicants, as they get exposed every day, especially through inhalation to an environment saturated by the toxicants for a long period for their occupational purposes. Inspite of having such extensive applications of calcium carbide in industrial field the experimental studies on its adverse effects are actually found to be negligible. In the light of this fact, our study is targeted to investigate the probable consequence of the exposure via inhalation calcium carbide by mimicking the situation of people who occupationally get exposed to this toxicant on regular basis.

MATERIALS AND METHODS

Materials: Calcium carbide and other fine chemicals were acquired from Sigma Aldrich, fluorescent tagged antibodies were obtained from Thermo Fisher Scientific whereas the primers were obtained from BioRad.

Animal maintenance: Male 4-6 weeks aged Swiss-albino mice (*Mus musculus*) with body weight 20-25g were maintained in a cross ventilated room with 12 hours cycle of light and darkness. Temperature of the room was maintained at 27°C^0 (± 2°C) with relative humidity of 44%-56% and the animals were provided with adequate supply of food and water. All of experimental procedures were executed following institutional animal ethical guidelines implemented by Institutional Animal Ethics Committee (IAEC), Rammohan College, Committee for Control and Supervision of Experiments on Animals (CCSEA), MoFAHD DAHD, Government of India. Minimum necessary number of animals were used for experimentation in order to minimize animal suffering and valid statistical evaluation.

Toxicant (CaC_2) exposure: Designated animals were grouped into two categories- control and toxicant exposed group. Exposed group of animals were further divided into four groups. Respective group of animals were exposed to 3gm, 5gm, 7gm and 9gm calcium carbide per day with requisite volume of water (w:v::1:10) for 15 minutes in a leak proof container of 24 litre volume. After 40 days of exposure the animals were sacrificed to perform the experimental procedures.

Real-time qPCR for Cox2 and PGES mRNA level: After obtaining total RNA from lungs of experimental animals, it was preserved in Trizol (ThermoFisher Scientific, Waltham, MA). First cDNA was acquired using iScript reaction mixture prepared with iScript Reverse Transcriptase, Nuclease free water and RNA template and then was incubated with reaction mixture in thermal cycler for 26 min (BIO-RAD, iScript cDNA Synthesis Kit). Next, the cDNA was amplified using the Sso Advanced Universal SYBR Green Supermix (BIO-RAD). Relative level of Cox2 and PGES were calculated with reference to GAPDH using the comparative CT (2_{CT}) method (Godfrey, 2009; Rao et al., 2013).

Flow cytometric analysis

Following the standard procedure of collagenase perfusion technique, the lung cells were isolated from the lungs of experimental animals.

To determine the percentage of apoptotic cells, the isolated pulmonary cells of control and exposed animals were suspended in 500µl 1X Binding Buffer and incubated with 5µl AnnexinV-FITC and PI for 15min at room temperature in dark. AnnexinV-FITC staining was analyzed by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2) (Chatterjee et al., 2019).

To evaluate the percentage of the T-cell subsets, first trypan blue exclusion assay was performed with the

isolated pulmonary cells to determine cell viability. The process was followed by washing and were treated with proteinase inhibitor. In T-cell subpopulation analysis, cells were incubated with fluorescence tagged CD3, CD4, IL17A (for TH17 analysis) and CD25 and FOXP3 (for Treg analysis) antibodies for 15 min at room temperature in dark atmosphere. Flowcytometric analysis and cell sorting were performed by using FACSAria™ (BD BioScience, Franlin Lakes, NJ). Acquisition was carried out against unstained cells by using CELL Quest software (BD Biosciences, Franlin Lakes, NJ) (Dong, 2009).

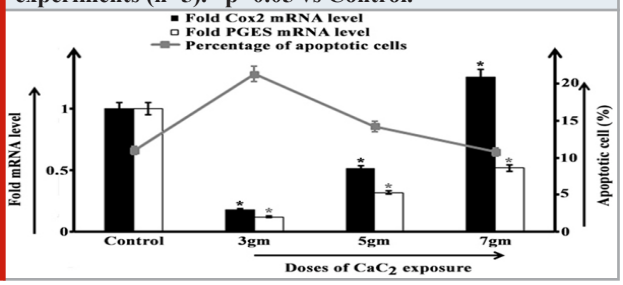
Statistical analysis: Conventional method was used for the calculation of means and SEM. Statistical differences among experimental groups were evaluated by the student's t-test. Data analysis was carried out using the GraphPad Instant software (GraphPad, La Jolla, CA, USA).

RESULTS AND DISCUSSION

In case of chronic inflammatory response, a crucial role is played by PGE2 which acts as an important mediator of chronic inflammation associated diseases (Wautier and Wautier, 2023). Biosynthesis of PGE2 is dependent upon two enzymes cyclooxygenase2 (COX2) and prostaglandin E synthase (PGES) (Gosset, Berenbaum and Levy, 2006). Therefore, in order to investigate the inflammatory conditions of lung tissue after scheduled toxicant exposure through inhalation, experimental animals were sacrificed and the levels of Cox2 and PGES mRNA had been observed through RT-PCR technique.

Data demonstrated a sharp decline in both Cox2 and PGES mRNA level after 3gm of CaC_2 exposure as compared to control group of animals. Parallelly, a significant increase in percentage of apoptotic cells than control group had been found in the afore-said group. In 5gm CaC_2 exposed group a moderate increase in Cox2 level than that in 3gm exposed group had been observed, though the value remained significantly lower than the control group. The mRNA level of Cox_2 had been found to increase steeply after 7gm CaC_2 exposure with even higher value than the unexposed animals.

Figure 1: Cox2 mRNA and PGES mRNA expression level (fold change) in lungs (bar diagram) and representation of apoptotic lung cells (line curve) isolated from control and 3gm, 5gm and 7gm calcium carbide exposed male mice respectively after 40 days of exposure. Data were expressed as mean+SD and were obtained from four independent experiments (n=5). *p<0.05 vs Control.



The PGES mRNA level in 5gm and 7gm CaC_2 exposed group had also elevated significantly than 3gm exposed group, though the value remained lower than the control group. But, both of 5gm and 7gm CaC_2 exposed group had exhibited a gradual declining pattern in percentage of apoptotic cells (Figure 1) as reported in our previous study (Banerjee et al., 2023).

Inflammatory and anti-inflammatory conditions in our body are balanced by two antagonistic subpopulations of T-cells, i.e., TH17 cells and regulatory T-cells (Treg cells) (Noack and Miossec, 2014).

In 3gm CaC_2 exposed group Cox2 mRNA level significantly declined than control while both of TH17 and Treg cell percentage escalated with greater percentage of Treg cells as observed in our experiment. In 5gm and 7gm exposed groups the Cox2 mRNA level demonstrated a surging pattern with steep increase in 7gm exposed group in parallel to gradual reduction in TH17 and Treg cell percentages (Figure 2).

Figure 2: Representation of CD3+/CD4+/IL17A+ TH17 and CD3+/CD4+/CD25+/FoxP3+ Treg cells (line curve) isolated from lungs of control and 3gm, 5gm and 7gm calcium carbide (CaC_2) exposed male mice after 40 days of exposure along with respective Cox2 mRNA level (bar diagram). Data were expressed as mean+SD and were obtained from four independent experiments (n=5). *p<0.05 vs Control.

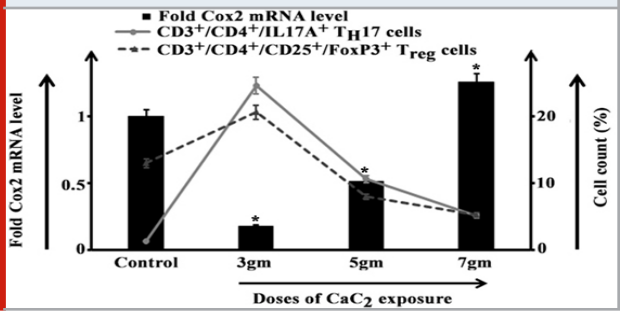
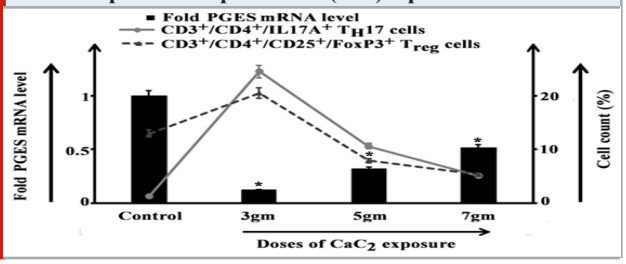


Figure 3: Relationship between CD3+/CD4+/IL17A+ TH17 and CD3+/CD4+/CD25+/FoxP3+ Treg cells (line curve) isolated from lungs of control and 3gm, 5gm and 7gm calcium carbide (CaC2) exposed male mice after 40 days of exposure and respective PGES mRNA level (bar diagram). Data were expressed as mean+SD and were obtained from four independent experiments (n=5). *p<0.05 vs Control.



After 3gm toxicant exposure though a marked reduction in the level of PGES mRNA had been observed, but TH17 and Treg cell percentages increased. An increasing pattern

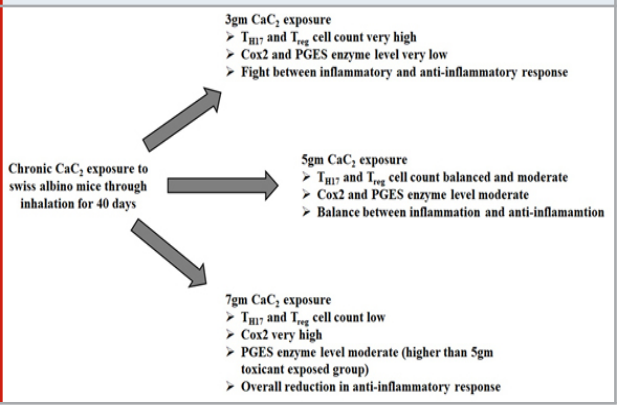
of PGES mRNA level had been in both of 5gm and 7gm exposed groups while approximate equivalent percentage of T_{H17} and Treg cells had been noted after 7gm CaC_2 exposure (Figure 3).

Upon lower grade of stress exerted by the exposure to toxicant (3gm CaC_2), the anti-inflammatory pathway probably got activated to balance the inflammatory situation of tissue. In order to accomplish this, anti-inflammatory Treg cell population had been augmented to suppress the inflammatory T_{H17} cell population which had been increased in response to exerted stress. The higher apoptotic cell percentage as an effect of fight between Treg and T_{H17} probably suppressed inflammation as demonstrated by low level of both Cox2 and PGES mRNA.

In 5gm toxicant exposed group, T_{H17} cell population still had been found higher than Treg cells. But all-over inflammatory condition had been reduced as demonstrated by the observed data. Although level of apoptosis had been decreased and therefore moderate inflammatory condition had been portrayed by somehow increased Cox2 and PGES mRNA level. Moreover, the reduced apoptotic cell percentage suggested a pro- to anti-apoptotic environmental shifting due to sustained inflammatory condition.

After 7gm CaC_2 exposure, T_{H17} cell population had been balanced by Treg cell population but percentage of apoptosis had been reduced further. As a result, towering augmentation of Cox2 with a significant increase in PGES mRNA level had been observed. This might lead to possible consequence of extensive cell damage due to overall reduction in anti-inflammatory response (Figure 4).

Figure 4: Diagrammatic status of inflammatory microenvironment of pulmonary tissue of male swiss albino mice after for 40 days of 3gm, 5gm and 7gm calcium carbide exposure through inhalation.



CONCLUSION

3gm CaC_2 exposure augmented cell death probably to combat the challenge imposed by stress, while cell death was reduced in 5gm and 7gm CaC_2 exposure indicating accumulation of altered and damaged cells. In order to recruit prostaglandin in site of damaged cell, Cox2 and PGES mRNA level augmented with increase in dose of exposure. Anti-inflammatory response was boosted up in 3gm and reduced in 5gm and 7gm CaC_2 exposure as reflected by the pattern of Treg and T_{H17} cell percentage. This disrupted inflammatory response likely due to the accumulation of damaged cells which reflected by the spike of Cox2 mRNA level after chronic exposure.

Author's contribution

KDC and PC were responsible for conceptualization and designing of the study. PG, SB and RG were responsible for model development, planning of experiments and data collection. KDC, PS, PG, SB and DP were responsible for result analysis and interpretation. All authors equally contributed in literature research, manuscript preparation, editing and review.

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Statement of ethics: This study was approved by the Institutional Animal Ethics Committee (IAEC), Rammohan College, affiliated under University of Calcutta, Kolkata, India (1795/PO/Re/S/14/CPCSEA, 28.02.2018 – 27.02.2023) vide approval number RMC/IAEC-2/1 dated 27.11.2021.

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Date : 31st June 2024

Place : Bhopal

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