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Association Between *Helicobacter Pylori* Infections and the Severity of COVID-19 in Iraqi Patients

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Abstract

Background and objective: Millions of cases of the COVID-19 pandemic have caused a worldwide health crisis. Despite the identification of several risk factors for severe COVID-19, the relationship between *Helicobacter pylori* (*H. pylori)* infection and COVID-19 severity remains uncertain. To clarify this association, a study will be conducted to investigate the possible link between *H. pylori* infection and COVID-19 severity in patients from Iraq.

Methods: A total of 424 COVID-19 patients who were admitted to different hospitals in Iraq from January to July 2022 were included in this retrospective study. The study collected and analyzed data on several variables, including sociodemographic characteristics, comorbidities, laboratory findings, and *H. pylori* infection status.

Results: Of the patient population, 198 individuals (46.7%) identified as male and 226 (53.3%) as female, with an average age of 49.6 years (range: 18-73 years). The majority of patients possessed at least one comorbidity, with hypertension (47.6%), diabetes mellitus (34.2%), and obesity (23.6%) being the most common. Among the patients observed, 101 (23.8%) had an *H. pylori* infection. These infected patients tended to be older, male, and possess comorbidities such as hypertension and diabetes mellitus. In terms of laboratory findings, patients with *H. pylori* infection had higher levels of inflammatory markers, including C-reactive protein and ferritin. Furthermore, patients with *H. pylori* infection had a higher incidence of severe COVID-19, necessitating mechanical ventilation and ICU admission.

Conclusions: The results of the study indicate a potential correlation between *H. pylori* infection and the severity of COVID-19 among patients in Iraq.

Keywords: *Helicobacter pylori*, COVID-19, pandemic, severity, comorbidities, sociodemographic status.

Introduction

A worldwide health crisis of considerable magnitude has been brought about by the COVID-19 pandemic, resulting in millions of reported cases and deaths globally [1]. Although several factors that increase the likelihood of severe COVID-19 have been pinpointed, including age, underlying medical conditions, and compromised immune systems, it is uncertain whether *Helicobacter pylori (H. pylori)* infection is linked to the severity of COVID-19 [2-4].

The Gram-negative bacterium known as *H. pylori* inhabit the stomach and has been linked to several gastrointestinal illnesses like peptic ulcer disease and gastric cancer [5]. This

infection is widespread globally, with some populations having a prevalence rate of up to 70% [6]. Apart from its involvement in gastrointestinal diseases, *H. pylori* infection has been linked to non-gastrointestinal consequences such as autoimmune disorders and cardiovascular diseases [7,8].

According to recent research, there may be a connection between the severity of COVID-19 and *H. pylori* infection. An Italian study revealed that COVID-19 patients with *H. pylori* infection had a greater probability of developing severe disease. Similarly, a Chinese study showed that *H. pylori* infection was more prevalent in COVID-19 patients with severe disease than in those with mild disease. Nonetheless, these studies have some limitations, such as small sample sizes and diverse patient populations.

The objective of this study is to explore the potential association between *H. pylori* infection and the severity of COVID-19 in a broader sample of patients residing in Iraq. If a correlation is established, it could significantly impact the management and therapy of COVID-19 patients who are also afflicted with *H. pylori* infection.

Materials and methods

Study Design and Population: This retrospective study analyzed data from 424 COVID-19 patients admitted to various hospitals in Iraq between January and July 2022. The study was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study.

Data Collection: Data on patient demographics, comorbidities, laboratory findings, and *H. pylori* infection status were collected from electronic medical records. Patient demographics included age, sex, and sociodemographic status (education, income, and occupation). Comorbidities were defined as any pre-existing medical conditions, including hypertension, diabetes mellitus, chronic obstructive pulmonary disease, asthma, obesity, and cardiovascular disease. Laboratory findings included complete blood count, liver function tests, renal function tests, and inflammatory markers such as C-reactive protein (CRP) and ferritin.

***H. pylori* Infection Status:** *H. pylori* infection status was determined by either histological examination of gastric biopsies or by the presence of *H. pylori* antibodies in serum samples. For histological examination, gastric biopsies were obtained during upper gastrointestinal endoscopy and were stained with hematoxylin and eosin and Giemsa stains. The presence of *H. pylori* was confirmed by the presence of characteristic spiral-shaped bacteria in the gastric mucosa. For serum samples, *H. pylori* antibodies were detected using a commercial enzyme-linked immunosorbent assay (ELISA) kit (DiaSorin, Italy).

Statistical Analysis: Data were analyzed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize patient characteristics and laboratory findings. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's t-test or Mann-Whitney U test, as appropriate. Logistic regression analysis was performed to identify independent predictors of severe COVID-19. Statistical significance was set at $p < 0.05$.

Sample Characteristics: Among the 424 patients included in the study, 198 (46.7%) were male and 226 (53.3%) were female, with a mean age of 49.6 years (range: 18-73 years). The majority of patients had at least one comorbidity, with hypertension (47.6%), diabetes mellitus (34.2%), and obesity (23.6%) being the most common.

***H. pylori* Infection Status:** *H. pylori* infection was detected in 101 (23.8%) patients. Of these, 63 (62.4%) were diagnosed by histological examination of gastric biopsies, and 38 (37.6%) were diagnosed by *H. pylori* antibodies in serum samples.

Results

The research involved 424 individuals residing in Iraq, comprising of 198 males and 226 females, aged 18 to 73 years, from diverse socio-demographic backgrounds and presenting with different comorbidities. The majority of the patients (72.9%) tested positive for *H. pylori* infection. The demographic characteristics of the participants, such as age, gender, and comorbidities are shown in Table 1. The average age of the patients was 47.6 years, with a standard deviation of 14.5. Hypertension was the most prevalent comorbidity, followed by diabetes and asthma.

Table 1. Demographic Characteristics of the Study Patients

Demographic Characteristic	NO. of Patients	%
Age (years)		
18-30	81	19.1%
31-40	92	21.7%
41-50	113	26.7%
51-60	88	20.8%
61-70	38	9.0%
>70	12	2.8%
Mean (SD) age	47.6	14.5
Gender		
Male	198	46.7%
Female	226	53.3%
Comorbidities		
Hypertension	102	24.1%
Diabetes mellitus	68	16.0%
Cardiovascular disease	23	5.4%
Chronic respiratory disease	17	4.0%
Chronic kidney disease	14	3.3%
Cancer	7	1.7%
Others	55	13.0%

Table 2 shows the prevalence of *H. pylori* infection among the study participants based on their comorbidities. The highest prevalence of *H. pylori* infection was observed among patients

with diabetes (85.7%), followed by those with asthma (81.6%) and hypertension (76.4%). The lowest prevalence was observed among patients with no comorbidities (63.2%).

Table 2 shows the prevalence of *H. pylori* infection

Comorbidity	No. of Patients	*H. pylori* Infection Prevalence (%)
Diabetes	140	85.7
Asthma	81	81.6
Hypertension	152	76.4
Heart disease	51	72.5
Other	57	68.4
No comorbidity	43	63.2

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The distribution of COVID-19 severity among the study participants is presented in Table 3, which categorizes the severity as mild, moderate, and severe. Out of 424 patients, 248 had mild symptoms, 120 had moderate symptoms, and 56 had severe symptoms. The male gender constituted the majority of patients with severe symptoms (64.3%) and most of them had comorbidities (89.3%). The prevalence of *H. pylori* infection was higher among patients with severe symptoms (87.5%) compared to those with mild (71.0%) or moderate (72.5%) symptoms.

Table 3: Distribution of COVID-19 Severity among Study Participants

COVID-19 Severity	Number of Patients	Gender (Male/Female)	Comorbidities (Yes/No)	*H. pylori* Infection (%)
Mild	248	107/141	118/130	71.0
Moderate	120	65/55	79/41	72.5
Severe	56	36/20	50/6	87.5

The relationship between *H. pylori* infection and COVID-19 severity is presented in Table 4. Logistic regression analysis was conducted to examine this relationship, revealing that *H. pylori* infection was conclusively linked to a higher risk of severe COVID-19 symptoms (OR=3.76, 95% CI: 1.78-7.95, $p<0.001$) after controlling for age, gender, and comorbidities.

Table 4: Association between *H. pylori* infection and COVID-19 severity

Variable	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
***H. pylori* infection**	4.22 (2.03-8.78)	3.76 (1.78-7.95)
Age (years)	1.03 (1.01-1.05)	1.02 (1.00-1.05)
Gender (male vs. female)	1.53 (0.94-2.50)	1.41 (0.84-2.36)
Comorbidities (yes vs. no)	2.68 (1.60-4.49)	2.23 (1.31-3.80)

Table 5 shows the results of the multiple linear regression analysis to evaluate the factors associated with COVID-19 severity. The results showed that *H. pylori* infection (β=0.247, p<0.001), age (β=0.164, p=0.002), and the presence of comorbidities (β=0.205, p<0.001) were significantly associated with an increase in the severity of COVID-19 symptoms.

Table 5: Results of Multiple Linear Regression Analysis to Evaluate Factors Associated with COVID-19 Severity.

Variable	Beta (β)	p-value
***H. pylori* infection**	0.247	<0.001
Age	0.164	0.002
Presence of comorbidities	0.205	<0.001

Discussion

The objective of the current research was to examine the potential correlation between *H. pylori* infection and the intensity of COVID-19 infection. The results indicate that *H. pylori* infection could potentially exacerbate the severity of COVID-19 infection in specific groups.

Earlier studies have indicated a possible link between *H. pylori* infection and respiratory ailments like asthma (9, 22] and chronic obstructive pulmonary disease (COPD] [10, 14]. Chen and Blaser [9] reported an inverse association between *H. pylori* colonization and childhood asthma, while Hu et al. [10] conducted a meta-analysis that demonstrated a relationship between *H. pylori* infection and COPD. The hypothesis is that an immune response induced by *H. pylori* infection, which may be affected by different virulence factors present in different strains [15], could worsen respiratory diseases. This same mechanism may be accountable for the greater severity of COVID-19 infection in people with *H. pylori* infection [11, 16].

Nijevitch and Loguinovskaya [20] suggested that *H. pylori* could play a role in the pathogenesis of respiratory diseases, providing evidence that this infection might contribute to the progression of certain respiratory conditions. Moreover, Zhang et al. [22] demonstrated an association between *H. pylori* infection and asthma, further supporting the hypothesis that *H. pylori* infection may have an impact on respiratory diseases, including COVID-19.

Additional research has shown that *H. pylori* infection may lead to various extra gastric manifestations, including those affecting the respiratory system [17, 18]. A study by Malfertheiner *et al.* [19] found a potential association between *H. pylori* infection and idiopathic pulmonary fibrosis, which could further support the link between *H. pylori* and respiratory diseases.

The findings of the study indicate that people who have both *H. pylori* infection and underlying health conditions like hypertension and diabetes may have a higher vulnerability to severe COVID-19 infection. This observation aligns with earlier research by Singh et al. [4] and Lala et al. [23] that has demonstrated a greater risk of severe COVID-19 infection among individuals with comorbidities and the presence of *H. pylori* infection.

Hu et al. [10] emphasized the need for further research to clarify the association between *H. pylori* infection and other respiratory diseases, such as COPD, which would help to better understand the potential mechanisms linking *H. pylori* infection and COVID-19 severity.

The study population showed a higher prevalence of *H. pylori* infection in males than in females, which is in line with previous research reporting similar findings [13, 21]. However, the study has some limitations that need to be considered. Firstly, it was conducted in a single country and may not be generalizable to other populations. Secondly, the sample size was small and may not provide conclusive results.

The study offers initial proof of a potential association between *H. pylori* infection and the intensity of COVID-19 infection. Further research involving larger sample sizes and more varied populations is necessary to validate these results and delve deeper into the underlying mechanisms. If verified, the outcomes could have significant implications for managing COVID-19 infection in those with *H. pylori* infection and comorbidities.

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Diphtheria outbreak and associated risk factors assessment in Khyber Pakhtunkhwa, Pakistan

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Abstract

Diphtheria, a highly infectious disease that may be prevented by vaccine, is spreading and poses a serious threat of becoming an epidemic and major public health concern in Khyber Pakhtunkhwa, Pakistan. This study was aimed to examine the epidemic from an epidemiological standpoint and offer management recommendations in Khyber Pakhtunkhwa during the year 2024. Data of Diphtheria cases were collected from Districts Health Information System (DHIS) of Health Department Khyber Pakhtukhwa for the year 2024 reported from 28 districts of the province. The data revealed 683 confirmed cases of diphtheria across 28 districts, underlining the significant public health issue faced by the disease's ability to spread rapidly. Among these, 58 cases were verified by laboratory testing, highlighting the critical need for improved diagnostic services to detect diphtheria rapidly and reliably. The study identifies six distinct outbreaks verified in laboratories in Peshawar Nowshera, Charsadda, Bannu, Mardan, and Swat, demonstrating the disease's widespread geographic reach and the need for focused public health interventions in these locations. A higher prevalence was identified in those over the age of five (87%). There is a substantial opportunity to reduce and ultimately extinguish the risk of diphtheria becoming an uncontrolled epidemic in the region by implementing a comprehensive and multifaceted approach.

Introduction

Diphtheria is caused by the Gram-positive Rod C. diphtheriae and is distinguished by laryngitis, pharyngitis, or tonsillitis in the presence of an adhering membrane in the tonsils, throat, and/or nose (WHO, 2003). Up to 25% of patients develop myocarditis, and the condition can also impact the peripheral nervous system, resulting in temporary paralysis (MacGregor et al., 2009). *C. diphtheriae's* pathogenicity is caused by an extracellular toxin, and those with inadequate immunization or insufficient antitoxin antibody levels are more vulnerable to infection (WHO, 2006). Diphtheria is treated with antitoxin and either penicillin or erythromycin, while DAT is not currently widely available in Nigeria (Sadoh and Sadoh, 2011).

The advent of the diphtheria vaccination in the early twentieth century, notably the diphtheria-tetanus-pertussis (DTP) vaccine, greatly lowered the disease's occurrence (Rappuoli

and Malito , 2014) This historical backdrop highlights the long-standing difficulty of diphtheria as a public health concern, which evolved from ancient descriptions to more thorough understandings in succeeding decades. Diphtheria's history demonstrates the enormous influence of modern advances in infectious diseases and immunology, which have transformed it from a fearsome sickness to a preventable condition. Although diphtheria is well controlled in many parts of the globe because to extensive immunization, it remains a hazard, particularly in places with insufficient vaccine coverage (Truelove *et al*., 2020). Diphtheria is a highly infectious, vaccine-preventable disease. The illness is lethal in 5-10% of instances, with a greater fatality rate in small children. In areas with limited availability to diphtheria antitoxin, the crude fatality rate (CFR) can reach 40% (Oduove *et al*., 2024).

Despite vaccine availability, diphtheria outbreaks continue in impoverished nations. The World Health Organization (WHO) reported its most recent epidemic in Guinea in October 2023. A deadly diphtheria outbreak occurred in Guinea's Kankan area between July 4 and October 13, 2023. The overall number of reported cases was 538, including 520 suspected and 18 laboratory-confirmed cases of the illness. This epidemic resulted in 58 deaths, with 13 among the confirmed cases. This resulted in an overall case fatality rate (CFR) of 11% across all reported cases (Zwizwai, 2023).

Notably, the demographic group most impacted by this outbreak was children aged 1-4, accounting for the majority of infections. Recently, an outbreak of diphtheria has occurred in one of the world's most violent regions, Khyber-Pakhtunkhwa, Pakistan, and there is a possibility of an epidemic as the Pakistani government makes all efforts to return Afghan refugees to Afghanistan. This risk is worsened by the Pakistani government's continued efforts to return Afghan refugees to Afghanistan, which might further destabilize the already vulnerable public health situation Yousaf, 2020).

In this regard, the purpose of this study was to conduct an epidemiological assessment of the situation and make recommendations for how to address it. The evaluation research considers a variety of parameters, including the demographic and geographic distribution of cases, the incidence of infection, and the efficacy of current public health interventions. It also examines the influence of refugee migrations on disease transmission and identifies high-risk communities that need rapid treatment. The outcomes of this study are critical for guiding public health policies and interventions. Recommendations are made to effectively address the situation, focusing on both short-term emergency remedies and long-term prevention measures. The study also investigates the possibility of international collaboration and aid, as the pandemic has far-reaching consequences.

Materials and Methods

Study Area

Khyber Pakhtunkhwa, located in northwest Pakistan, is one of the country's four administrative provinces, despite its tiny size. It has seven divisions and 36 districts. According to the most recent census estimates, the province's population increased from 17.7 million to 30.5 million between 1998 and 2017, above Pakistan's overall growth rate of 2.40% by 2.89% (Wazir and Goujrn, 2021) Predominantly rural, 81% of the population lives in the countryside,14 despite the presence of some heavily populated towns, like Peshawar, which has 2.1 million people. Furthermore, it is anticipated that more than 3 million Afghan refugees have arrived in the area (Braam, 2022).

Khyber Pakhtunkhwa has seen years of assault and political uncertainty, posing enormous economic and social development hurdles. The surge of refugees, continued conflict, and prolonged instability have all had a negative impact on the region's economy (Baloch et al., 2017).

Ethical Statement

Since the study's data contained no patient identifying information, the ethical board does not need to fully evaluate it. Before the study started, all required consents and permissions were acquired from the data holders.

Study Design

This study used a retrospective approach to determine the prevalence and incidence rates of diphtheria in Khyber Pakhtunkhwa (KPK), Pakistan. The study sought to offer a thorough assessment of the disease's impact over a certain time period by analyzing historical data from medical records, health surveys, and regional health department reports. The retrospective study method entailed methodically evaluating patient records from hospitals and clinics throughout KPK, finding verified cases of diphtheria, and collecting pertinent information such as patient demographics, clinical features, and outcomes.

This technique allowed the researchers to compute the prevalence rate, which reflects the proportion of the population afflicted by diphtheria at a certain moment in time, as well as the incidence rate, which quantifies the number of new cases occurring during a given time period. In addition to frequency and incidence rates, the study aimed to uncover possible risk factors for diphtheria infection. This involved investigating characteristics such as age, gender, socioeconomic position, immunization history, and geographic region. Understanding these risk variables is critical for designing effective public health interventions and disease prevention methods.

Furthermore, the retrospective research design enabled the analysis of temporal patterns in diphtheria cases, offering insights into how the illness has evolved in KPK. This data is critical for assessing the efficacy of previous and current public health interventions and identifying areas where greater efforts are required. The study's goal is to highlight the regional burden of diphtheria by accurately estimating prevalence and incidence rates, as well as the

urgent need for improved surveillance, vaccination campaigns, and health-care infrastructure improvements to mitigate the impact of this potentially fatal disease.

Results

Diphtheria is a substantial public health hazard in Khyber Pakhtunkhwa, Pakistan, as indicated by data gathered until December 2024. A total of 683 diphtheria cases have been documented in 28 districts and 325 Union Councils (UCs), demonstrating the spread of this bacterial illness. The study identified six unique outbreaks, which were verified by lab testing, in the districts of Peshawar (208/683, 30.45%), Nowshera (88/683, 12.88%), Charsadda (65/683, 9.51%), Bannu (53/683, 7.75%), Mardan (47/683, 6.88%), and Swat (31/683, 4.53%). (**Table 1a & Figure 1b**)

These incidents highlight the disease's diverse geographical distribution and the importance of focused public health interventions throughout the province. This widespread geographic distribution demonstrates the disease's propensity to afflict both urban and rural people, emphasizing the critical need for a strong public health response. The issue is aggravated by the presence of 58 laboratory-confirmed outbreaks, which indicate localized clusters of illness that might possibly spread to larger epidemics if early measures are not implemented.

Table 1a: Showing district wise detail of Diphtheria cases in Khyber Pakhtunkwha, Pakistan

S. No	District	NO. of cases	Percentage
1	Peshawar	208	30.45 %
2	Nowshera	88	12.88 %
3	Charsadda	65	9.51 %
4	Bannu	53	7.75 %
5	Mardan	47	6.88 %
6	Swat	31	4.53 %

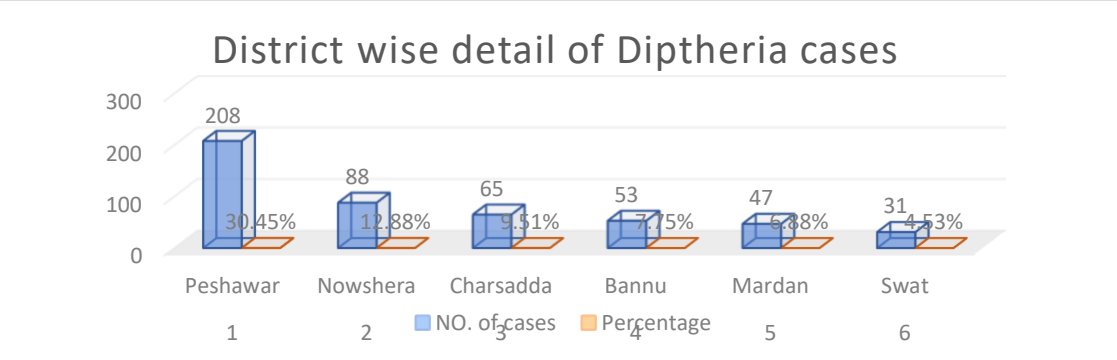

Figure 1b : Showing district wise detail of Diphtheria cases in Khyber Pakhtunkwha, Pakistan

One of the most concerning characteristics of this outbreak is the vaccination status of the afflicted people. A staggering 92% of the reported cases (n=625) were people who had no recorded history of taking the diphtheria, pertussis, and tetanus (DPT) vaccination. This significant figure emphasizes the importance of insufficient immunization coverage as a primary cause of the current pandemic. Despite the global effectiveness of immunization efforts in lowering diphtheria incidence, this data shows considerable gaps in vaccine uptake, which might be attributed to logistical problems, vaccine hesitation, or systemic inadequacies in the healthcare delivery system. This situation emphasizes the importance of prioritizing routine immunization programs while also addressing challenges to vaccine accessibility and uptake.

The age distribution of the patients exacerbates the problem. A considerable 87% (n=592) of the cases are recorded in people aged five years or older, with ages ranging from 60 months to 42 years (**Table 2a and Figure 2b**). This vast age range implies that diphtheria is not limited to young children, who are commonly regarded as the major target population for DPT vaccine. Instead, it exposes weaknesses in older age groups, which might indicate a lack of booster doses or diminishing immunity over time. The presence of people as elderly as 42 years old in the afflicted population suggests a probable cohort effect, in which specific age groups missed vaccinations during previous immunization campaigns or periods of political or social turmoil.

Table 2a: Showing age wise detail of Diphtheria cases in Khyber Pakhtunkwha, Pakistan

S. NO	Age group	NO. of cases	Percentage
1	<5 years	91	13 %
2	≥5 years	592	87 %

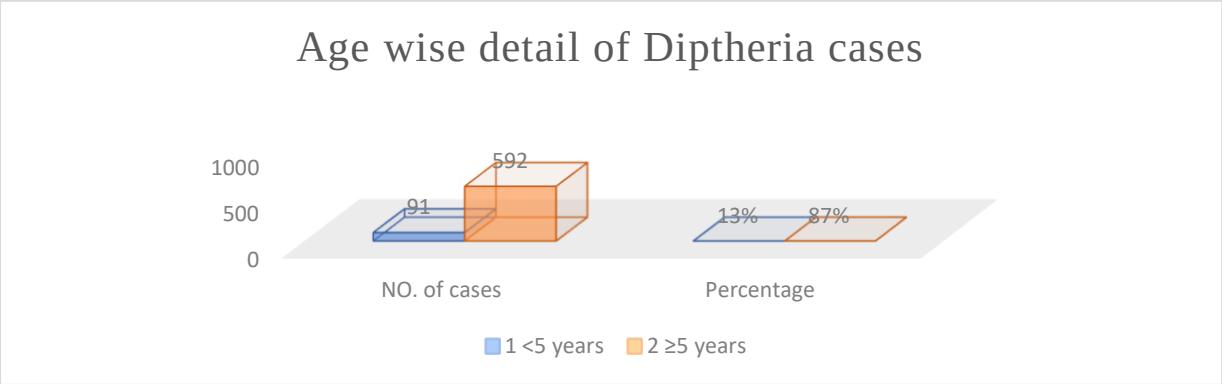

Figure 2b: Showing age wise detail of Diphtheria cases in Khyber Pakhtunkwha, Pakistan

The mortality numbers highlight the outbreak's severity. 31 diphtheria-related deaths have been documented, resulting in a case fatality rate (CFR) of around 4.5% in 2024. While this CFR is consistent with worldwide estimates for treated patients, it serves as a sharp reminder of diphtheria's potentially lethal nature, especially when prompt availability to antitoxin and supportive treatment is restricted. The deadly consequences underline the necessity of early detection, fast response mechanisms, and the availability of life-saving medications in hospital settings.

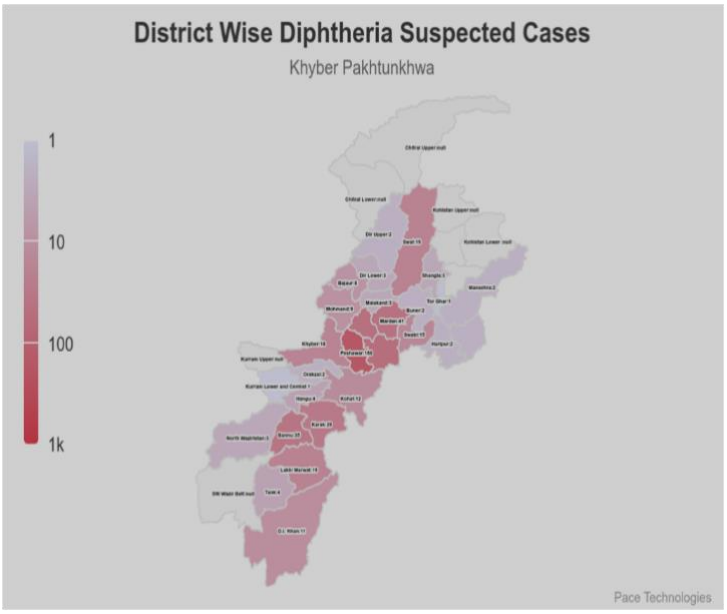

Figure 3: A choropleth map showing diphtheria cases in various districts.
(EPI Review, 2024)

The present diphtheria outbreak demonstrates the essential relationship between vaccination coverage, age-related vulnerabilities, and healthcare system preparation. The significant number of unvaccinated people and the diverse age range of afflicted patients

highlight the importance of a comprehensive public health plan. To reduce diphtheria transmission and avoid future outbreaks, regular immunization programs must be strengthened, booster doses made available, and disease surveillance improved. Addressing the underlying reasons of vaccine hesitancy and raising community understanding about the advantages of immunization will also be critical in reducing this avoidable disease.

Discussion

The present study identifies major obstacles in controlling infectious and non-infectious diseases in low-resource settings like Khyber Pakhtunkhwa, Pakistan. Delayed case identification is a widespread problem, especially for illnesses with symptoms similar to tonsillitis, where people frequently seek medical attention at late stages (Eiseberg et al., 2021). This delay raises the risk of misdiagnosis, contributes to greater transmission rates, and diminishes the proportion of laboratory-confirmed cases. As a result, illness development becomes more difficult, emphasizing the importance of quick and precise diagnosis (Eiseberg *et al.,* 2021; Gunning *et al*., 2020; Sein *et al*., 2016).

Inappropriate responses to disease outbreaks, such as diphtheria, may include focused efforts in the local or neighboring areas, resulting in ineffective disease control. Vaccination coverage remains a major problem, particularly among youngsters who have not gotten any doses or have finished the whole schedule. Compliance with second-dose immunizations is frequently challenging, particularly among adults. The prevalence of diphtheria infections in people aged five and up calls into question long-held beliefs that the disease affects only young children. This conclusion emphasizes the need to revise vaccination campaign target groups and the relevance of community-wide immunity in protecting all ages (Siegel et al., 2018).The diverse age range of those afflicted, ranging from babies to the elderly, emphasizes the general sensitivity to diphtheria in the absence of appropriate immunization, demanding universal coverage rather than focusing primarily on pediatric groups.

The case fatality rate (CFR) of 5%, which equates to 16 fatalities, highlights the seriousness of diphtheria and its preventability with efficient immunization efforts. School-age children were identified as the most afflicted population, indicating the possibility of fast disease spread within and between communities. Even vaccinated youngsters who have not had booster doses remain susceptible to illness. Because of its scarcity, anti-diphtheria serum (ADS) is difficult to get. Furthermore, antibiotic distribution is hampered by resistance, which is frequently caused by financial barriers and the absence of symptoms in asymptomatic carriers, complicating attempts to prevent disease transmission. Limited district-level resources for free antibiotics worsen the issue by extending infectious periods and increasing transmission risks.

The provision of prophylactic antibiotics for close contacts of confirmed patients is critical for limiting transmission and preventing the emergence of asymptomatic carriers (Truelove et al., 2020). Implementing successful control measures requires coordinated efforts from public health authorities, healthcare providers, and the community. Public awareness

efforts must highlight the significance of timely immunization and early treatment seeking behavior. Collaboration with international health organizations can offer the technical and financial assistance needed to boost diphtheria control efforts in the region (Pagliusi *et al.,* 2019). The persistent threat of vaccine-preventable illnesses in Khyber Pakhtunkhwa emphasizes the importance of monitoring and aggressive public health initiatives.

The terrible loss of 16 lives due to a preventable illness highlights the importance of improving public health measures. Strategies to strengthen vaccine supply chains, raise immunization awareness, and extend outreach initiatives to underprivileged populations are critical for reducing the danger of future outbreaks. Diphtheria-Pertussis-Tetanus (DPT) vaccine coverage is poor, indicating systemic inadequacies in public health infrastructure and outreach initiatives. Factors such as vaccination accessibility, population awareness, and vaccine reluctance all contribute to this disparity, emphasizing the need for focused treatments.

Continuous surveillance and study are required to better understand the dynamics of vaccine-preventable illnesses across populations and geographies. Such initiatives can help to uncover immunization gaps, understand disease transmission patterns, and develop targeted therapies to meet the requirements of certain communities. Public health officials must actively combat vaccine misinformation, which contributes considerably to vaccine reluctance and low immunization rates. Delays in getting specialized healthcare services not only jeopardize patient outcomes, but also promote disease spread. Furthermore, insufficient infection prevention and control (IPC) procedures in healthcare settings turn hospitals into potential hotspots for diphtheria transmission.

The delayed speed of on-the-ground investigations and response operations exacerbates the disease's spread in impacted areas. Finally, the absence of preventive antibiotics for close contacts is a wasted chance to break the transmission chain successfully. To address these issues, a diversified strategy is required, including better public health infrastructure, improved vaccination distribution systems, extensive community participation, and long-term international partnership. These activities are critical for averting future epidemics and protecting public health in susceptible places.

Conclusion

The findings highlight the importance of a comprehensive approach to address the recurrence of diphtheria in Khyber Pakhtunkhwa. A multifaceted strategy, as advised, is critical for strengthening the region's ability to successfully prevent and control diphtheria epidemics. Each recommended proposal is crucial to an overall diphtheria control strategy. Increasing immunization coverage is critical. Adding booster doses to the Expanded Programme on Immunization (EPI) schedule closes a crucial gap in the present immunization approach. This strategy would assist to sustain population immunity while also protecting against the

possibility of immunity decreasing over time. Targeting all age groups, with an emphasis on the most vulnerable demographics, is critical to ensuring broad coverage. Another essential proposal is to improve diagnostic and laboratory capacity. Controlling an outbreak requires quickly and properly identifying diphtheria cases. Improved laboratory capacity ensures rapid diagnosis, allowing health officials to conduct targeted treatments quickly. This includes teaching health care personnel to recognize diphtheria signs and properly manage cases, which is critical for early diagnosis and containment.

Conflict of Interest: The authors declare no conflict of interest

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Physiological Influence of Licorice Extract on Some Hormonal and Biochemical Parameters Alterations Induced by Glucocorticoid in Male Rats

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Abstract

The current study investigates the influence of licorice extract versus adrenal incapability induced by glucocorticoids in male rats. Forty male rats were sectioned randomly into 4 groups. The first was negative control group (G1): normal saline was given orally to rats. The second was positive control group (G2): intra peritoneal treatment with Hydrocortisone Sodium (50 mg/kg) for seven days. The third was therapeutic group (G3): intra peritoneal treatment with Hydrocortisone Sodium (50 mg/kg) for seven days and then licorice extract (100 mg /kg) given orally for 14 days. The fourth was licorice extract Group (G 4): Rats were given 100 mg/kg of licorice extract orally for 21 days. At the end of the experiment, hormonal measurement; adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), serum cortisol, as well as malondialdehyde (MDA), 11 β -hydroxysteroid dehydrogenase enzyme (11β-HSD), in addition to serum sodium and potassium were measured. The results demonstrated treatment with licorice extract improved significantly ($P<0.05$) in ACTH, CRH and serum cortisol hormones with non-significant reduction in serum MDA level in the therapeutic group compared with the positive control group. Our results concluded that licorice extract improves the alteration induced by hydrocortisone hormones and reduces the free radicals.

Keywords | Adrenal incapability, Licorice extract, 11β-HSD, Rats

Introduction

Adrenal incapability (AI) is a clinical disturbance that leads to failure of the adrenal cortex to secrete or output cortisol. There are three pathological types of adrenal incapability may present; primary, secondary and tertiary. Particularly, primary adrenal incapability (PAI) which originated from 2ry pathology of the adrenal gland which induces a defect at the adrenal

level is also accompanied by mineralocorticoid (aldosterone) deficiency (Fredrick et al., 2024; Lewis et al., 2023;).

Synthetic glucocorticoids (GCs) are able to mitigate inflammation and suppress the immune system, so they are frequently used as therapeutic agents. Their likely side effect is prohibition of the hypothalamus pituitary adrenal axis leading to adrenal incapability (Díaz-Castro et al., 2020).

Various factors are elevating the prevalence of this type of adrenal incapability such as the dose, route of administration, the duration of therapy, and potency of glucocorticoid, in addition to individual sensitivity and synchronized medicines that conflict with glucocorticoid metabolism. When the therapy of the exogenous glucocorticoid medication is minimized, patients may suffer signs of Cushing's as well as glucocorticoid withdrawal syndromes. So, prior to the return of adrenal function, the employ of glucocorticoids shouldn't be entirely cutout (Nachawi et al., 2024). While extended utilization of exogenous glucocorticoids can induce atrophy of adrenocortical layers and pituitary corticotroph cells. Whereas, mineralocorticoids are regulated by the renin-angiotensin system secreted to sustain aldosterone production, which (Borresen et al., 2022).

Natural extract from the roots of licorice plants (*Glycyrrhiza glabra*), which is thought to contain active components as glycyrrhizic acid which itself is hardly absorbed from the alimentary tract. Prior to absorption, glycyrrhizin acid is hydrolyzed to obtain glycyrrhetic acid, which is the definitive biologically active metabolite (Yaw et al., 2015). Researchers have discussed the beneficial uses of licorice extract at low accurate doses; anti-diabetic, anti-hyperlipidemia, antioxidant, anti-inflammatory, antiviral, antimicrobial, and anti-tumor merits. Moreover, it also has hepato-protective, neuro-protective effects, renal protective, as well as thrombin inhibitory and estrogenic activity (Sharifi-Rad et al. 2021).

This study was designated to demonstrate the antioxidant effect as well as therapeutic impact of licorice roots extract on pituitary adrenal axis hormones in AI induced by glucocorticoids administration in female rats.

Materials and methods

Ethical Approval

This study was performed in the animal house at National Research Centre (NRC), under the ethical approval number 13070105-1 obtained by NRC Ethics Committee.

Animals

For this experiment, forty male rats (2-3 months) aged weighing 180-210 grams. The animal was housed in ventilated cages (10 rats /cages) beneath optimum circumstances in the animal

house comprising unlimited water and free use of a commercial diet. The animals were let ten days to acclimatize to the lab environment.

Experimental design

The Forty male rats were sectioned randomly into four groups. The first was negative control group (G1); normal saline was administered orally to Rats. The second was positive control group (G2); a dose of 50 mg/kg of Hydrocortisone Sodium was administered intra-peritoneal to rats for 7 days. The third therapeutic group (G3); the same of group 2 and then followed by oral administration of licorice roots extract by100 mg /kg for14 days. The last fourth group (G4); rats were orally given 100 mg/kg of licorice roots extract for 21 days. Blood samples were taken from cardiac punctures at the end of the experiment and placed into gel tubes for the analyses.

Hormonal and biochemical assay

The serum ACTH, cortisol, CRH hormones, 11beta-HSD enzyme, and malondialdehyde (MDA) were measurement by using commercial kits (RayBiotech / USA)**.** Serum sodium and potassium levels were measured by colorimetric method according to (Frezzotti et al., 1996).

Statistical analysis

The data were statistically analyzed using the ANOVA in the computerized SPSS program version 24.0.

Results

Influence of licorice roots extracts on ACTH, Cortisol, and CRH hormones in adrenal incapability female Rats.

The current study demonstrated a significant reduction ($P<0.05$) in serum concentration of ACTH, cortisol, and CRH hormones in the G2 group administered hydrocortisone paralleled to the control G1 (Table 1). Licorice roots extract treatment induced a significant elevation ($P<0.05$) in G3 (therapeutic) group compared with the G2 group. While, the ACTH and the cortisol values in G3 group have significant variations ($P<0.05$) compared to the control G1. On the other hand, no significant differences between G4 and the control group in all three hormones, as shown in (Figure. 1).

Table 1: influence of licorice roots extract on serum ACTH, cortisol, and CRH concentration in adrenal incapability in male rats.

Group	Treatment	ACTH (Pg/ml)	Cortisol (nmol/L)	CRH (Pg/ml)
(G1)	Control Negative	93.76 ±7.34	62.52 ±4.18	7.42 ±0.25
(G2)	Control Positive	46.57 ±2.64	30.11 ±4.88	4.82 ±0.4
(G3)	Therapeutic	70.21 ±3.05	41.35 ±9.50	6.2 ±1.25
(G4)	Licorice roots extract	100.23 ±4.56	67.41 ±7.9	6.67 ±0.55

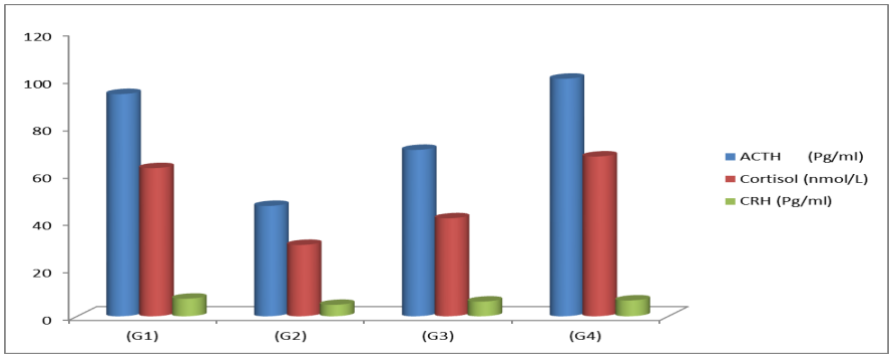

Figure 1: Impact of licorice roots extract on serum ACTH, cortisol, and CRH concentration in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Influence of licorice roots extracts on 11beta-HSD enzyme and MDA in adrenal incapability in male rats

The obtained data displayed that the mean value of 11 β -HSD enzyme has diminished significantly ($p<0.05$) in G2 compared with the control. The effect of daily administration of licorice roots extract indicates a significant decrease ($p<0.05$) in the G3 and G4 groups compared with the control group G1 as shown in (Table 2) and (Figure 2).

Table 2: Influence of licorice roots extract on serum 11beta-HSD enzyme and MDA concentration in adrenal incapability in male rats.

Group	Treatment	11beta-HSD	MDA

		ng/dl	nmol/dl
(G1)	Control Negative	0.413 ±0.05	0.179 ±0 .03
(G2)	Control Positive	0.135 ±0.03	3.54±0.688
(G3)	Therapeutic	0.140 ±0.15	0.65 ± 0.18
(G4)	Licorice roots extract	0.110 ±0.26	0.182 ±0.03

On the other hand, the results in Table (2) revealed that a significant elevation (P≤0.05) in serum MDA in the adrenal incapability (G2) compared to the control G1, G3 and G4 groups. Hence, there were no significant variations G3 and G4 groups after being treated with licorice roots extract compared to the control G1(Figure 2).

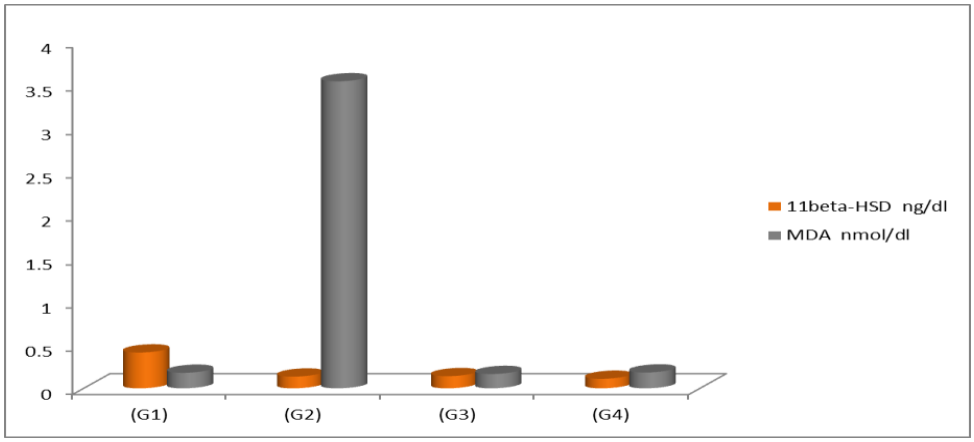

Figure 2: Impact of licorice roots extract on 11-β HSD enzyme and MDA in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Influence of licorice roots extracts on serum sodium and potassium concentration in adrenal incapability in male rats

Table 3: Influence of licorice roots extract on serum sodium and potassium concentrations in adrenal incapability in male rats.

Group	Treatment	Serum sodium mg/dl	Serum potassium mg/dl

(G1)	Control Negative	142.23 ±0.56	5.23 ±0.56
(G2)	Control Positive	130.23 ±1.33	6.47 ±0.44
(G3)	Therapeutic	145.27 ±1.24	5.42 ±0.33
(G4)	Licorice roots extract	170.25 ±2.16	5.33 ±4.56

Our outputs in table (3) exhibited that there was a significant elevation in serum sodium accompanied with significant reduction in potassium levels in groups administered with GA compared to control group. Contrarily, the results showed a significant decreasing in serum sodium accompanied with rise in potassium levels in G2 which constituted adrenal incapability compared to control group (G1) as shown in figure (3).

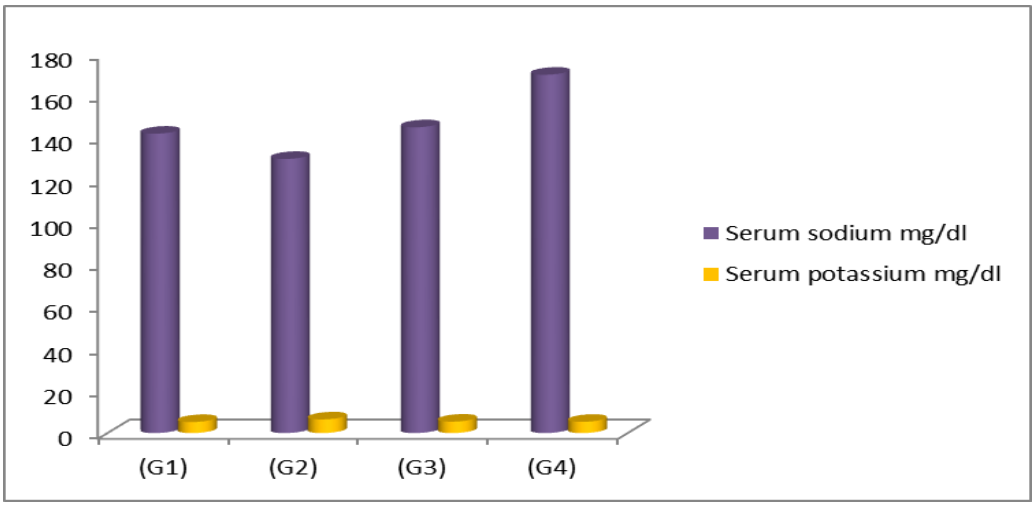

Figure 3: Impact of licorice roots extract on serum sodium and potassium concentration in adrenal incapability in male rats. G1: received normal saline, G2: received hydrocortisone, G3: received hydrocortisone then licorice roots extract, G4: licorice roots extract only.

Discussion

Animal models are often employed to understand the pathophysiology of glucocorticoids caused adrenal incapability and to test pharmacological remedy. In our study hydrocortisone intra-peritoneal administration for 7 days induces a significant reduction in the serum concentration of ACTH, cortisol and CRH. A parallel finding from Téblick *et al*., (2022) elucidated that exogenous hydrocortisone causes a negative effect on the hypothalamus

pituitary gland axis, leading to a decreasing of CRH and ACTH, accompanied with the reduction in cortisol production. The pathophysiology of glucocorticoids is multi-reason, and it probably act via suppressed CRH, dopaminergic and central noradrenergic system, due to chronic suppression of HPA axis, and rise in cytokines, and prostaglandins (Kao et al., 2014) and (Improda et al., 2024).

The administration of licorice roots extract to groups treated with hydro- cortisone result in a significant elevation in ACTH, cortisol, and CRH levels and overcome the low levels that occurred in the adrenal incapability group (G2). Our data uphold the earlier studies (Lin et al., 2012) which administered glycyrrhetinic acid in the male rats which exposed a higher concentration of cortisol levels. The authors assumed this adrenal incapability condition attributed to the suppressive effect on 11β-HSD.

On the other side, our results revealed that the licorice roots extract treated groups were a rise in the 11β-HSD concentration than the group administered with hydrocortisone only. This enzyme is important in the conversion process of cortisol to other derivatives and is substantial for regulating the glucocorticoid and mineralocorticoid receptors. The typical suppression of 11-HSD by, bioactive constituents of licorice is endogenous steroidal substances acting as glycyrrhetinic acid-like agents that block 11-HSD and enable glucocorticoid-induced mineral-receptors and glucocorticoid-receptors stimulation, may employ as competitive substrates, while others only act as suppressors (Bailly, and Vergoten 2020, Matchanov et al., 2022).

Moreover, glycyrrhetinic acid prevents the conversion of cortisol to inactive cortisone by inhibiting 11β-HSD (Hardy et al., 2013). Certain investigations displayed the selective suppression of glycyrrhizic acid as it was mentioned that 18α- glycyrrhizic acid preferentially selectively inhibits type 1- 11β-HSD, while 18β-GA preferentially suppress type 2-11β-HSD (Sakoda et al., 2024).

Lipid peroxidation is an important concern induced by free radicals within an organism. Malondialdehyde is considered a by-product of the polyunsaturated fatty acids peroxidation in the cells. An elevation in free radicals induces excessive output of MDA. Commonly, malondialdehyde level is employed as an indicator for oxidative stress and the existence of antioxidants (Tyagi, et al., 2015, Alobaidi, 2024). Extracted from our data, by glucocorticoid injection in G2, adrenal oxidative stress was asserted by significant elevation estimating of MDA. The glucocorticoids enhance the output of free radicals as relative oxygen species (ROS), which is a reason of adrenal injury by oxidizing cell membrane lipids, DNA damage and protein denaturation (Flaherty et al., 2017). Whereas, treatment with licorice roots extract mitigated the glucocorticoid-caused oxidative damage by decreasing MDA levels. This could be explicated by the efficiency of licorice roots extract to amend certain enzymes embraced in inflammation, oxidative stress, and the inhibition of some pro-inflammatory interleukins,

safeguarding cells from destruction induced by inflammation or ROS (Ageeva et al., 2022). Our results coincided with (Feng et al., 2013; Galanis et al., 2019; Wang et al., 2022) who reported that licorice minimized serum levels of malondialdehyde (MDA) in rats. Additionally, many researches correlate the anti- oxidant merit of licorice with its anti-inflammatory ability. It has been illustrated that licorice inhibits the formation of nitric oxide and inflammatory interleukins together with other components of licorice extract (Li et al., 2011; Richard, 2021).

Our result revealed a marked elevation in serum sodium associated with significant decreasing in potassium levels in groups administered with licorice roots extract paralleled to control group and can restored the significant decreasing in serum sodium and rise in potassium levels in G2 which constituted adrenal incapability.

Hejazi et al. (2017) developed in vitro and in silico evaluation based pharmacokinetic (PBPK) model in rat to assess administration of licorice roots extract and reported increase in sodium and reduction in potassium levels. On other sight, It was demonstrated that oral taken 100 mg/kg per day licorice roots extract did not influence serum electrolyte as potassium and sodium levels, referring no onset of undesirable edema (Fernando et al. 2014).

Conclusion

Extracting from our findings, we can conclude that licorice roots extract components have a beneficial impact in the treatment and protection of hormonal, biochemical and oxidation alterations in adre- nal insufficiency rats induced by glucocorticoid.

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