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Head lice; Tamarindus indica; Trigonella foenum; Acacia concinna; anti-lice lotion; stability analysis.

Preparation and Evaluation of Antimicrobial Activity of Herbal Lotion: Potential Anti-lice Activity

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ABSTRACT: Head lice are ectoparasites that live on the human head. The present study aimed to formulate an organic anti-lice lotion containing extracts of Tamarindus indica, Trigonella foenum, and Acacia concinna, as well as tea tree and neem oils. The ethanolic and aqueous plant extracts were obtained by Soxhlet extraction and the decoction method. Oil-in-water emulsion was evaluated for physical appearance, spreadability, pH, viscosity stability, FTIR analysis, and antimicrobial activity. F12 was selected due to its smooth appearance, optimum spreadability, stable pH, and optimum viscosity. Stability studies showed that the F12 formulation was physically and chemically stable. All the functional groups were verified to identify the active ingredients, and their presence in the final formulation proved its compatibility. The disc diffusion method was used to test the antibacterial activity of ethanolic extracts as well as optimized formulations against two bacterial species, namely Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli). F12 formulation showed an inhibitory effect (zones of inhibition 10.34±1.2 and 9.82±0.87). In conclusion, organic anti-lice lotion offered a potential treatment strategy due to its anti-lice application, suggesting it may be an alternative treatment for head lice problems.

1. INTRODUCTION

Head lice are a very common problem throughout the world. Head Lice (*Pediculus Humanus capitis*) are ectoparasites that are found on the human scalp and suck human blood through their saliva, having vasodilating properties[1]. This problem is very common in school children aged between 4 and 13 years, both male and female. The major cause is unhygienic conditions [2]. Approximately 12 million people were treated in the USA, of whom most of the patients are children [3]. The mode of

transmission is direct, and indirect transmission is through sharing hats, caps, pillows, brushes, and combs [4]. People having problems with head lice have several clinical manifestations, which include pruritus, lymphadenopathy, local erythema, scalp impetigo, papules, and, in severe cases, anemia. Head lice not only cause physical problems, but it also causes psychological distress [5].

Head lice cause serious bacterial infections. It contains bacteria known as *Acinetobacter baumannii,* which causes meningitis, pneumonia, and Endocarditis [6,7]. It may also contain *Bartonella quintana,* which causes Trench fever. These are severe bacterial infections that may lead to death [8,9].

Ivermectin is an antiparasitic drug that is used to treat both endo- and ectoparasite infections, while permethrin is an insecticide, but their use is associated with several side effects such as rash, burning sensation, irritation, itching, dandruff, stinging, dryness, swelling, and redness on the skin, etc [10,11]. To overcome these problems, different natural remedies are used against head lice with minimal harmful effects. Even though natural remedies are less potent than synthetic treatments in some scenarios [12].

Tamarindus indica is a medicinal plant also known as tamarind, which belongs to the family Fabaceae. It has very amazing effects against certain diseases such as jaundice and liver diseases, also used as a laxative, skin cleanser, and to treat yellow fever. It has antimicrobial as well as antioxidant activity and is used to treat obesity [13]. *Acacia concinna* is a medicinal plant that is also known as Shikakai. The antimicrobial role of plant-derived extracts has been very well observed [14]. It has very vast uses for medical and commercial purposes. The most important uses in hair care are for dandruff and are safe for the skin as well[15]. It has very strong activity against head lice. It is used as an analgesic, insecticide, and antimicrobial agent [16]. *Trigonella foenum* is a medicinal plant belonging to the Leguminosae family, which is widely used in the medical field. It is used as an anti-cancer, anti-diabetic, anti-microbial, cholesterol-lowering agent, and as an anti-parasitic agent [17,18]. The use of plant extracts has been widely used in cosmetics, especially for sensitive skin[19,20]

The present study aims to develop an organic anti-lice lotion containing natural extracts and oils with marvelous anti-parasitic properties, based on the above literature. Different natural or synthetic polymers are used to provide the essential structural network, which is required for a lotion. The main challenge that we are addressing is the ability of the lotion to treat the condition with fewer harmful effects.

2. MATERIALS AND METHODS

2.1 Materials

Acacia concinna fruit, *Trigonella foenum-graecum fruit,* and *Tamarindus indica* fruit were obtained from the local market of Lahore. All other solvents and chemicals, such as ethanol, liquid paraffin, lanolin alcohol, beeswax, cetosteryl alcohol, Tween 80, tea tree oil, neem oil, almond oil, castor oil, and argon oil, used in this research were of analytical grade.

2.2 Methodology

5% ethanolic extract of *Tamarindus indica*, *Acacia concinna,* and *Trigonella foenum* by the Soxhlet extraction method [21]. Briefly, fruits of *Trigonella foenum, Tamarindus indica,* and *Acacia concinna* were packed in filter paper. Filter paper containing the compound was placed inside a thimble chamber. The Soxhlet extractor was placed onto a flask. The flask contained absolute ethanol. The condenser was equipped with a Soxhlet apparatus. Ethanol was heated to 78 °C, vaporizing into the sample thimble. These vapors entered the condenser, condensed back into liquid, and dripped back into the siphon arm. The liquid in the siphon arm dripped back into the flask, and the process was continued. After that, the solvent was evaporated by a rotary evaporator, and the extract was collected [22-25].

The standard decoction method was used to prepare 5% aqueous extract of *Trigonella foenum*, *Tamarindus indica,* and *Acacia concinna* [26]. 5 g of powder from each plant was weighed and placed in beakers containing 95 ml of distilled water. These beakers were placed on a tripod stand and heated. The heating process continued until they reached a boil. After that, they were cooled down at room temperature and filtered using Whatman filter paper [27-29].

2.3 Preparation of Oil-in-water Emulsion Base

The standard method was used to prepare oil-in-water emulsions, formed with beeswax, cetosteryl alcohol, and lanolin alcohol [30]. These chemicals were taken in a 100 mL beaker and heated until they melted. Then, liquid paraffin was taken into another beaker. The previously melted solution was taken and mixed with liquid paraffin. Solution A was formed. In another beaker,

distilled water was taken and mixed with Tween 80. Solution B was formed. After that, solutions A and B were mixed using a homogenizer to form a primary emulsion. Then add natural ethanolic and aqueous extracts and natural oils were added at different proportions to each emulsion base and stirred continuously until the base formed a good lotion [31,32]. The schematic diagram for preparing the organic anti-lice lotion is shown in **Figure 1**. The formulation design is shown in **Table 1.**

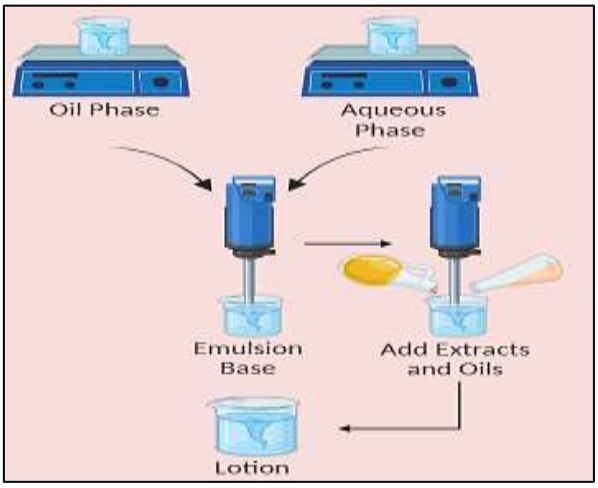

Fig. 1. Schematic diagram for the preparation of an organic anti-lice lotion.

Table 1. Concentrations of ingredients for various formulation designs.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Liquid Paraffin	35.0	35.0	35.0	35.0	35.0	35.0	36.5	35.0	36.5	36.1	36.5	29.1
Beeswax	2.3	2.3	2.3	2.32	2.3	2.3	2.4	2.3	2.4	2.4	2.4	1.9
Cetosteryl Alcohol	4.6	4.6	4.6	4.6	4.6	4.6	4.8	4.6	4.8	4.8	4.8	3.8
Lanolin Alcohol	1.5	1.5	1.5	1.5	1.5	1.5	1.6	1.5	1.6	1.5	1.6	1.2
Tween 80	1.5	1.5	1.5	1.5	1.5	1.5	1.6	1.5	1.6	1.5	1.6	1.2
Ethanolic Extract of *Tamarindus indica*	5	-	-	-	-	-	-	-	-	-	-	5
Ethanolic Extract of *Acacia concinna*	-	5	-	-	-	-	-	-	-	-	-	5
Ethanolic Extract of *Trigonella foenum*	-	-	5	-	-	-	-	-	-	-	-	5
Aqueous Extract of *Tamarindus indica*	-	-	-	5	-	-	-	-	-	-	-	-

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Aqueous Extract of *Trigonella foenum*	-	-	-	-	5	-	-	-	-	-	-	-
Aqueous Extract of *Acacia concinna*	-	-	-	-	-	5	-	-	-	-	-	-
Tea tree oil	-	-	-	-	-	-	1	-	-	-	-	1
Neem oil	-	-	-	-	-	-	-	5	-	-	-	5
Almond oil	-	-	-	-	-	-	-	-	1	-	-	-
Argon oil	-	-	-	-	-	-	-	-	-	2	-	-
Castor oil	-	-	-	-	-	-	-	-	-	-	1	-
Dis-Water QS to	100	100	100	100	100	100	100	100	100	100	100	100

2.4 Characterization of Lotion

2.4.1 Physical Appearance

The physical appearance of the lotion was inspected. The samples were placed between the thumb and index finger, and then the homogeneity and texture characteristics of the developed moisturizing formulation were assessed [33].

2.4.2 Spreadability

The spreadability of lotion on the skin plays a very important role in its effectiveness. It was observed by keeping a small amount of lotion between two glass plates. The upper glass plate was weighed at 1-minute intervals. The diameter of the spreading sample was measured, and the results were calculated.

2.4.3 pH

The normal skin pH ranges from 5.5 to 6.5. To determine the pH of the lotion, an electronic pH meter (Mettler Toledo pH-101) was used. A 10 mL sample was separated, and the pH meter was immersed in it. Three readings were taken, and the average pH was calculated [34].

2.4.4 Viscosity

The viscosity of the lotion was determined by a Brookfield viscometer. The viscosity of the lotion was measured at room temperature (25 °C). Three readings were taken, and the average reading was calculated.

2.4.5 Fourier Transform Infrared Spectrophotometer (FTIR)

The FTIR (Bruker) was used to check the composition and compatibility between the extract and the polymers. The FT-IR spectrometer was used in the region 4000-400cm-1 by employing the standard KBr pellet technique.

2.4.6 Stability Studies

2.4.6.1 Thermodynamic Stability

To determine the thermodynamic stability of the lotion, stress testing was conducted, including centrifugation, freeze-thaw cycles, and heating and cooling. The lotion was centrifuged at 3500 rpm for 30 minutes. The freeze-thaw cycle was performed under specific conditions (40°C and 25°C for 24 hrs). The heating and cooling cycle was performed under specific storage conditions (45°C and 4°C for 48 hrs) [35].

2.4.6.2 Physical Stability

To determine physical stability, the lotion was stored under specific conditions (40 ± 2°C/ 75 % ± 5% RH and 25 ± 2°C/ 60 % ± 5% RH) for 30 days to assess the overall stability of the developed formulation system. All the parameters were observed very carefully, such as pH, color, Odor, appearance, consistency, phase separation, and homogeneity [36].

2.4.7 Antibacterial Activity

Two bacterial strains were selected for antibacterial activity. *Staphylococcus aureus* (*S. aureus*) (ATCC 43300) and Escherichia coli (E. Coli) (ATCC 25922) were obtained from Sigma-Aldrich and used to examine the antimicrobial activity of plant extracts. The disc diffusion method was employed to determine the zone of inhibition. Both bacterial specimens were inoculated into Mueller-Hinton Broth and incubated for 24 h at 37 °C. Bacterial suspensions of each strain were collected and then spread onto nutrient agar plates. Paper discs 5mm in diameter were prepared from Whatman No. 1.5 filter paper and placed on the culture media. The inhibitory activity of the F12 formulation was tested by adding 10 µl of the formulation to 5mm paper discs. Distilled water was used as a negative control, and the streptomycin disc was used as a positive control. The Petri plates were incubated at 37 °C for 20-24 hours. A digital vernier caliper was used to measure the zone of inhibition. The method was repeated in triplicate.

2.4.8 Statistical Analysis

The analysis of variance (one-way ANOVA) using the GraphPad Prism program was applied to analyze the experimental data of different treatment groups.

3. RESULTS AND DISCUSSION

3.1 Physical Appearance

The physical appearance of all formulations was inspected. It was found that they had a very smooth texture; the dispersion was homogeneous and had a very appealing appearance, with no sign of phase separation.

Fig 2. Physical appearance of formulation F12.

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3.2 Spreadability

The spreadability of lotion on the skin is considered to play a very important role in its effectiveness.

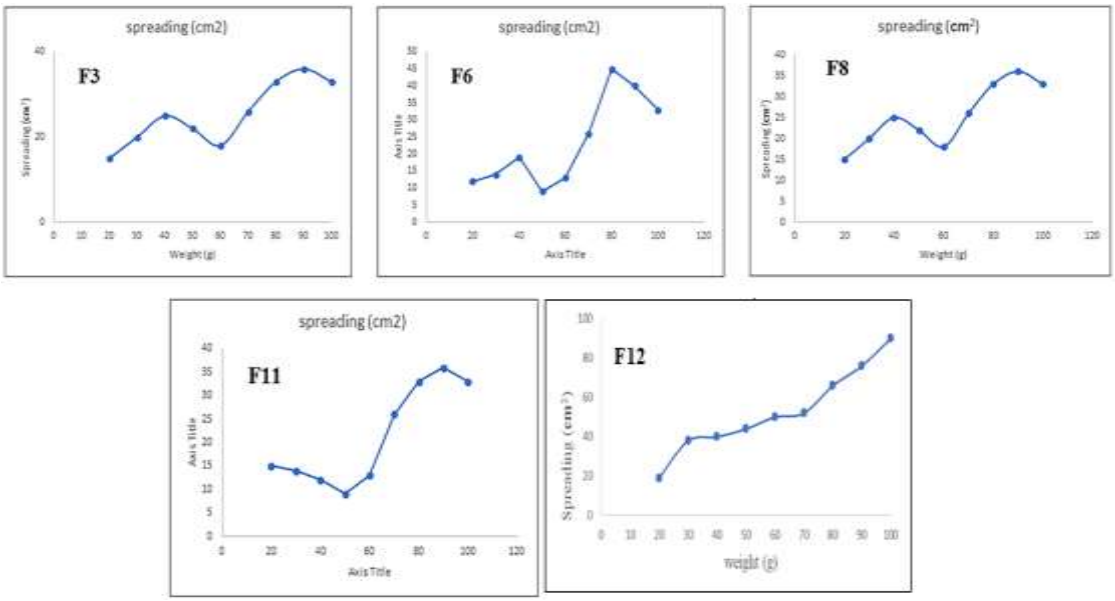

Fig. 3. Measurement of spreading of F3, F6, F8, F11 and F12 Formulations

3.3 pH

The pH of some formulations was too acidic, while others were too basic; formulation F12 showed an optimal pH.

3.4 Viscosity

The viscosity of the lotion was determined by a Brookfield viscometer. The viscosity of the lotion was observed, and it was found that the viscosity was 310.34 under a shear stress of 5.6 Pa. Thus, the lotion showed Newtonian flow.

3.5 Fourier Transform Infrared Spectrophotometer (FTIR)

The IR spectra of the optimized lotion were observed. The FTIR spectra of 5% ethanolic extracts, tea tree oil, and F12 are shown in Fig. 4. FTIR for Tamarindus indica showed peaks between 1382.25 and 1652.24, which showed the presence of (S=O) sulfate group and (C=C) alkene stretching. The peaks between 879.49 and 1087.30 showed the presence of C=C and (C-O) primary alcohol. The peaks at 2833.40 and 2974.88 showed the presence of (C-H) aldehyde and (C-H) alkane stretching [37]. The functional groups of *Trigonella foenum* were obtained from FTIR spectra. The peaks at 2833.40–2974.68 are assigned to both symmetric and asymmetric stretching of methyl (-CH3) and methylene (-CH2) groups, which are attributed to the existence of fatty acids and their methyl esters within the carboxyl groups (COOH). The sharp peak at 3328 showed the presence of (C-H) alkyne stretching [27]. For *Acacia concinna*, the peak at 3300 cm^{-1} is assigned to (OH) stretching. A moderate peak at 2924 cm^{-1} was attributed to the (C-H) stretching, while the two peaks at 1407 and 1324 cm^{-1} were due to the (CH_2) and (C–O–C) stretching. A peak at 1024 cm^{-1} was attributed to the (C-O) stretching of cellulose[38].

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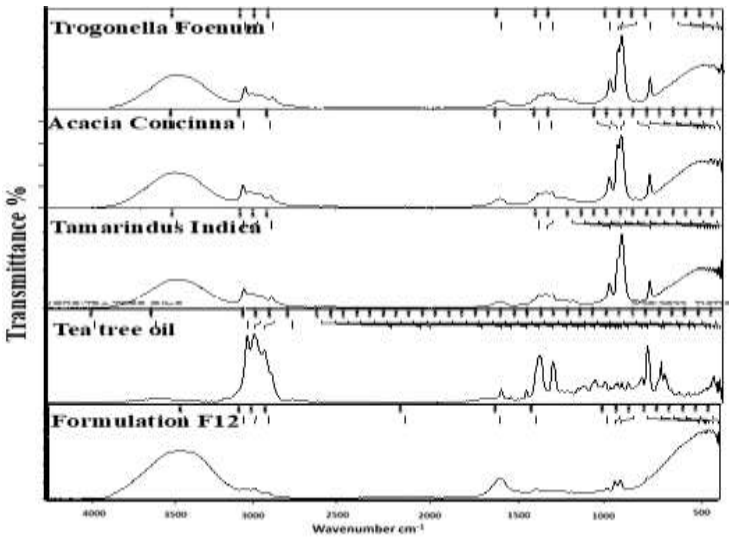

Fig. 4. FTIR analysis of Trogonella Foenum, Acacia Concinna, Tamarindus Indica, Tea tree oil, and F12 formulation.

3.6 Stability Studies

3.6.1 Physical Stability

The physical stability of the lotion was determined by observing physical appearance, homogeneity, and phase behaviour. After the stability studies, it was observed that the lotion's integrity was retained and showed no signs of instability.

Table 2. Physical stability indicating appearance, color, Phase separation, and Homogeneity.

Parameter	Condition	W0	W1	W2	W3	W4
Appearance	25±2°C/60% ± 5% RH, 40±2°C/75% ± 5% RH	Thin Lotion	Thin Lotion	Thin Lotion	Thin Lotion	Thin Lotion
Colour	25±2°C/60% ± 5% RH, 40±2°C/75% ± 5% RH	White to off-white color	White to off-white color	White to off-white color	White to off-white color	White to off-white color
Phase separation	25±2°C/60% ± 5% RH, 40±2°C/75% ± 5% RH	No	No	No	No	No
Homogeneity	25±2°C/60% ± 5% RH, 40±2°C/75% ± 5% RH	Yes	Yes	Yes	Yes	Yes

3.6.2 Thermodynamic Stability

To determine the thermodynamic stability of the lotion, various stress tests were performed, including centrifugation, freeze-thaw cycles, and heating and cooling cycles. The developed formulation did not exhibit any instability, such as creaming, precipitation, or phase separation. No coalescence or cracking was observed.

Table 3. Zone of inhibition (mm) of various ethanolic extracts, F12 formulation, and streptomycin.

Treatment groups	Zone of Inhibition (mm)	
	S. aureus	***E. coli***
Ethanolic Extract of *Tamarindus indica*	5.1±1.3	3.2±0.7
Ethanolic Extract of *Acacia concinna*	4.5±1.6	2.4±0.8
Ethanolic Extract of *Trigonella foenum*	3.6±0.6	5.2±1
F12 Formulation	10.34±1.2	9.82±0.87
Streptomycin	14.23 ±2	13.4±1.6

Data were presented in the form of three replicates ±SD.

3.7 Antimicrobial Activity

Zones of inhibition of ethanolic extracts of *Tamarindus indica, Acacia concinna, and Trigonella foenum, F12 formulation,* and streptomycin were tested against *S. aureus* and *E. coli* Table 3.

The physical appearance of the formulation is very important, as it reveals its properties. The physical appearance of the final formulation was very clear, with a smooth texture and homogeneous dispersion. It also showed no sign of phase separation [36]. It was observed that the spreadability of formulations F3, F6, F8, and F11 did not show optimum behavior, but formulation F12 showed very good and optimum behaviour of spreadability and was found to be the best for topical application Figure 3, [34]. The normal range of scalp pH is 5.5, and the hair shaft pH is 3.67[39]. The pH of the formulation plays a very important role in topical formulations. It can cause serious problems if the pH of the formulation does not match the pH of the skin[40]. Too acidic or too basic pH can cause serious problems. The pH of the final formulation was checked and found to be very close to the skin pH. The rheology of the lotion is very important. The lotion's viscosity was checked and found to be within the optimum range. The viscosity was 310.34 with the shear stress of 5.6pa [36].

In the FTIR analysis of tea tree oil, the peak at 2965 cm^{-1} was attributed to the (C–H) stretching vibration. The peak at 1127 cm^{-1} was due to the (C–O) stretching vibration of the tertiary alcohol. The peak at 910 cm^{-1} corresponded to the bending vibration absorption peak of an unsaturated double bond [41]. In the FTIR of blend formulation F12, all functional groups were identified, confirming the presence of the active ingredients and demonstrating the final formulation's compatibility. The slight change in intensity of the bending and stretching peaks demonstrated that no such physical interactions occurred in the blend formulation.

Stability studies of a formulation are a critical part of developing a new formulation. The stability studies of the final formulation were conducted. The final formulation was assessed for thermodynamic and physical stability. The results showed that the formulation was stable under stress conditions and showed no signs of instability [42]. The statistical analysis was conducted using SPSS (Statistical Package for Social Sciences). Based on ANOVA, it was found that the formulations were significantly different from each other, and the results were considered significant at the 5% level of significance ($p<0.05$). The post hoc test determined that differences among formulations were due to viscosity and spreadability. The F12 was chosen as the optimized formulation among all due to its optimal spreadability and viscosity.

During the incubation period, the extract samples were diffused from the discs into the medium, creating circular inhibition zones [43]. The herbal extract exhibited better antibacterial activity with no side effects [44]. The results showed that the F12 formulation exhibited a higher zone of inhibition against S. aureus (10.34±1.2mm), whereas against E. coli it was lower (9.82±0.87mm). The antibacterial activity was confirmed by the formation of a zone of inhibition [45]. The antibacterial activity of ethanolic extracts was not as effective as the commercial antibiotic streptomycin (14.23 ± 2 mm and 13.4 ± 1.6 mm) and was significantly lower. However, higher concentrations may be required to demonstrate potential antibacterial activity, which

also needs further safety evaluation. The combination of extracts showed a significantly larger zone of inhibition than the extract alone.

4. CONCLUSION

The current study revealed that the organic anti-lice lotion, containing extracts of Trigonella foenum, Tamarindus indica, and Acacia concinna, as well as essential oils such as tea tree and neem oil, was evaluated for physical appearance, spreadability, pH, viscosity, FTIR, and stability. Moreover, the antibacterial activity of the ethanolic extracts was detected against both *S. aureus* and *E. coli*. Among all the formulated lotions, F12 showed the best results. The results show that the formulation has potential as a topical treatment for head lice. Further studies are required to explore this formulation as an effective anti-lice product. Hence, it may be concluded that F12 is a potential formulation for people with head lice.

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AUTHOR CONTRIBUTIONS

Muhammad Irfan Siddique: Conceptualization, Supervision, Investigation.

Muhammad Ahmad: Methodology, Laboratory Analysis.

Qurat-ul-ain Shoaib: Data Curation, Formal Analysis.

Bandar Theyab Alenezi: Formal Analysis and Writing.

Howayada Mahany Mostafa: Formal Analysis and Writing.

Salma Yousif Sidahmed Elsheikh: Formal Analysis and Writing.

Mohd Imran and Abida Khan: Writing-Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available in this paper.

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