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COMPREHENSIVE SAFETY ASSESSMENT OF POLYHERBAL UNANI OINTMENT

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ABSTRACT

WHO mandates a safety evaluation on herbal medications before any research is done to prevent toxicity due to the material found in the soil and the environment. The recent study was aimed to evaluate safety parameters of polyherbal Unani ointment aimed to be used in treatment of the patients of hemorrhoids. It comprises determination of heavy metals, aflatoxin, pesticidal residue and microbial load. The study revealed the presence of microbial load and heavy metals like lead, cadmium, mercury and arsenic within the permissible limit as per WHO guidelines, while aflatoxins and pesticides residues were found to be absent in the sample. From this study, it was concluded that the polyherbal ointment was safe and free from toxic contaminants and microbes. Drug was found to be free from toxic matter which further can be used safely and effectively in preclinical and clinical trials and may be used as a therapeutic agent.

KEYWORDS: Safety study, herbal medicine, Unani, WHO guidelines, ointment.

INTRODUCTION

Herbal remedies have been utilized for centuries to address various health conditions, and the use of medicinal plants has greatly contributed to global health improvements. Despite the progress of modern medicine in recent years, plants remain a vital part of healthcare. In the last decade, there has been a notable increase in interest in medications derived from higher plants, especially phytotherapeutics.[1] Safety is a crucial aspect of quality control in the production and distribution of herbal remedies and other healthcare products. A common

misconception is that natural substances are risk-free. However, herbal remedies can have side effects, some of which may be harmful.[2] To ensure the safety of herbal drugs, safety studies must be carried out in accordance with WHO guidelines. These studies involve assessing levels of pesticide residues, heavy metals, aflatoxins, and microbial contamination. The presence of microorganisms in herbal medicines can diminish their effectiveness and lead to spoilage. Therefore, determining the extent of microbial contamination in the drug sample is crucial.[3] Pollution from factories, leaded gasoline, pesticides, fertilizers, soil composition, and other sources can contribute to the presence of heavy metals in herbal medicines. Contamination during manufacturing may occur through the use of lead-containing containers, grinding tools, or other equipment. Additionally, Asian herbal medicines (AHMs) may contain heavy metals if the plants are grown in soil with high levels of contamination.[4] Mycotoxins in plant materials can pose health risks both in the short and long term. Aflatoxins B1, G1, B2, and G2 are toxic secondary metabolites produced by fungi such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. Aflatoxins are considered some of the most potent naturally occurring carcinogens, primarily affecting the liver. The International Agency for Research on Cancer (IARC) has classified aflatoxins G1, B2, and G2 as possible human carcinogens (group 2 B), while aflatoxin B1 is classified as a human carcinogen (group 1).[5-7] Agricultural practices such as spraying, soil treatment during cultivation, and the use of fumigants during storage can result in the buildup of pesticide residues in medicinal plant parts. Additionally, pesticide substances carried by the wind may contaminate crops of medicinal plants growing in nearby fields.[7] Given the widespread global use of herbal medicines, it is essential to assess the potential risks associated with their use, particularly in terms of population exposure. The safety of herbal medicines remains a critical public health concern.[8] Hemorrhoids are a common anorectal condition characterized by the symptomatic enlargement and downward displacement of the normal anal cushions. This condition is a significant medical and social issue affecting millions of people worldwide. According to the National Institute of Health, the prevalence of hemorrhoids is 4.4% among individuals aged 45–60 years, with an estimated 50–86% of people globally experiencing hemorrhoids. In India, the condition affects 30–40% of the target population. While surgery can be an effective treatment for hemorrhoids, it is typically reserved for severe cases due to the risk of complications. In contrast, non-surgical treatments, especially those involving topical or pharmaceutical approaches, are often not fully effective.[9-10] Therefore, to deal with the current situation, a preclinical study is aimed to be conducted on the hemorrhoidal model of rat to assess the efficacy of Unani drugs in the

management of hemorrhoidal diseases. Many single and compound drugs are commonly used to treat hemorrhoids, but there has been limited research conducted to evaluate the scientific and therapeutic effects of these drugs in managing the condition. In order to create a novel and innovative treatment for hemorrhoids, medicinal plants from classical Unani literature were investigated and Unani herbal Ointment was prepared using single drugs namely, *Euphorbia thymifolia* L. (Doodhi Khurd), *Quercus infectoria* Olivier (Mazu) and *Allium ascalonicum* Linn. (Tukhm-e-Gandhana). These drugs are mentioned in literature to have pharmacological action of *Qabiz* (Astringent), *Muhallil-E-Warm* (Anti-inflammatory), *Habis-e-Dam* (Hemostatic), *Dafa-e-taffun* (Antiseptic), *Mullaiyin* (Laxative), *Mushil* (Purgative), *Mujaffif* (Siccative), *Musaffi-e-Dam* (Blood purifier), *Dafa-e-Kirm shikam* (Anthelmintic), *Jali* (Detergent), *Dafa-e-Tashannuj* (Antispasmodic), *Jazib* (Absorbent), etc.[11-17] The present study aims to evaluate the safety profile of an herbal ointment intended for use in the Unani system of medicine for treating hemorrhoids. The powdered ingredients were subjected to safety screening according to WHO guidelines before being used in preclinical research with an animal model of hemorrhoids. This step is crucial, as ensuring the quality of plant-based drugs is a prerequisite for their further evaluation of biological activity.

MATERIALS AND METHODS

Collection and Sample Preparation

Tukhm-e-Gandhana was sourced from Asia Enterprises, Khari Baoli, Delhi; *Mazu* from Dawakhana Tibbiya College, A.M.U, Aligarh and *Doodhi Khurd* was directly collected from the herbal garden of the Department of Ilmul Advia, AMU, Aligarh. The samples were carefully identified based on the morphological features described in both Botanical and Unani literature, and their authenticity was confirmed by the Pharmacognosy section of the Department of Ilmul Advia, A.M.U., Aligarh. They were further authenticated by Prof. Wazahat Hussain from the Department of Botany, A.M.U., Aligarh. The crude drugs were cleaned to remove any undesirable substances, such as dirt and foreign material, then dried in sunlight and powdered using an electric grinder. The powdered samples were sifted through a No. 80 sieve to ensure uniform particle size and stored in an airtight container for further analysis. Specimens of all three drugs used in the formulation were submitted to the Mawalid-e-Salasa Museum of the Department of Ilmul Advia (Unani Pharmacology), Faculty of Unani Medicine, A.M.U. Aligarh, and their respective voucher numbers are SC-408/24 (*Doodhi Khurd*), SC-413/24 (*Mazu*), and SC-414/24 (*Tukhm-e-Gandhana*). The powdered samples were then analyzed for the presence of heavy metals, pesticide residues, aflatoxins,

and microbial load and the result was determined in the AGSS Analytical and Research Lab (P) Ltd., Delhi. India. [ULR No. TC121152400009266F Report No. AGSS/AP/24071300007 Sample Dated 13/07/2024, Reported on 18/07/2024]

A. Microbiological determination tests

Total viable aerobic count (TVC)

The total viable aerobic count (TVC) of the test drug was performed to assess the antibacterial activity of the drug, following the specified test procedure.

Pre-treatment of the test drug

The herbal drug sample was dissolved using an appropriate method based on its nature, and any antimicrobial activity in the sample was neutralized or diluted. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G, Hi-media Labs, Mumbai, India), was used for diluting the test sample.

Plate count for bacteria

One milliliter of the pre-treated test sample was added to approximately 15 mL of liquefied casein-soybean digest agar in a 90 mm diameter Petri dish, ensuring the temperature did not exceed 45°C. Alternatively, the test sample was spread onto the surface of the solidified medium. Two dishes were prepared with the same dilution, inverted, and incubated at 30-35°C for 48-72 hours, unless a more reliable count was obtained in a shorter time. The number of colonies formed was counted, and the results were calculated using the plates with the highest colony count, up to a maximum of 300.[18]

Plate count for fungi

In a 90 mm diameter Petri dish, 1 mL of the pre-treated test sample was mixed with approximately 15 mL of liquefied Sabouraud glucose agar containing antibiotics at a temperature not exceeding 45°C. Alternatively, the test sample was spread onto the surface of the solidified medium. Two plates were prepared using the same dilution, inverted, and incubated at 20-25°C for 5 days, unless a more accurate count was obtained in a shorter period. The number of colonies that formed was counted, and the results were calculated using the plates with fewer than 100 colonies.[18]

B. Determination of Heavy metals

The heavy metal test is designed to assess the level of metallic impurities in the test drugs. Contamination of medicinal plant materials with arsenic, lead, mercury, and cadmium can occur due to various factors, including environmental pollution. These heavy metals, such as lead, mercury, arsenic, and cadmium, were identified in the test sample using ICP-MS.

C. Estimation of Aflatoxins

Sample preparation: The presence of aflatoxins B1, G1, B2 and G2 in the test sample of the given herbal drug powder was detected with LC-MS/MS. The test drug sample (2gm) and 60% acetonitrile/water (20 ml) were mixed for 2 minutes at high speed. The combined sample was centrifuged at 1600 rpm for ten minutes. The supernatant was collected and diluted with 2ml filtrate in 48 ml of Phosphate Buffered Saline (PBS, pH 7.4) to produce a solvent concentration of 2.5% or less; methanol/water was prepared with 2 ml of test sample diluted with 14 ml of Phosphate Buffered Saline (PBS, pH 7.4) to get a solvent concentration of 10% or less. At a flow rate of 5 ml/min, the sample diluent was run over the immunoaffinity column. The column was then rapidly blown with air to dry after being rinsed with 20 ml of distilled water at a flow rate of around 5 ml/min. The sample elute was mixed with 1.5 ml of distilled water. A sample volume of 500 µl was injected into the LCMS-MS. By comparing sample peak heights or areas to the total aflatoxin standard, the concentration of aflatoxin in the sample was determined.[19]

D. Pesticide residue

Medicinal plant materials may contain pesticide residues that accumulate from agricultural practices, such as spraying, soil treatments during cultivation, and the use of fumigants during storage. The test to assess specific pesticide residues, including organochlorine compounds, organophosphorus compounds, and pyrethroid compounds, was conducted using GC-MS/MS.[20]

RESULTS AND DISCUSSION

The microbial load determination shown in Tables 1 and 2 indicated that the total bacterial count was 48,000 cfu/g, while the total fungal count was 340 cfu/g. Specific pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, and *Pseudomonas aeruginosa*, were absent. The heavy metal analysis presented in Table 3 showed that metals such as arsenic, lead, mercury, and cadmium were below the limits of quantification (BLQ). Additionally, total aflatoxins (B1 + G1 + B2 + G2) and aflatoxin B1, as depicted in Table 4,

as well as pesticide residues, shown in Table 5, were found to be absent in the test sample. Studies on the safety and effectiveness of Unani medicines provide scientific validation for their traditional use. Since medicinal products directly impact an individual's health, ensuring safety is paramount when it comes to herbal drugs. The World Health Organization (WHO) stipulates that every finished product, whether a single or compound drug, must undergo a safety profile evaluation. Due to various environmental factors, medicinal plants can be associated with a wide range of microbiological contaminants, such as bacteria, fungi, and viruses. These contaminants can significantly affect the overall quality of herbal products and preparations. Therefore, a safety study was conducted to assess microbiological load, pesticide residues, aflatoxin contamination, and heavy metal contamination (including lead, mercury, cadmium, and arsenic).

Table 1: Microbial load in Ointment.

S. No.	Microbes	Result (CFU/g)	Permissible Limit
1.	Total Bacterial Count	48000	Not more than $1x10^5$ CFU/g
2.	Total Fungal Count	340	Not more than $1 x10^3$ CFU/g

Table 2: Test for Specific Pathogens in Ointment.

S. No.	Pathogens (/gm)	Result (gm)	Permissible limits
1.	*E. coli*	Absent	Absent
2.	*Salmonella*	Absent	Absent
3.	*P. aeruginosa*	Absent	Absent
4.	*S. aureus*	Absent	Absent

Table 3: Heavy Metal in ointment.

S. No.	Test Parameters (mg/kg)	Results (mg/kg)	Permissible limits (mg/kg) as per API	Method of Testing
1.	Lead (Pb)	BLQ (0.05)	Not more than 10.0	ICP-MS
2.	Arsenic (As)	BLQ (0.05)	Not more than 3.0	ICP-MS
3.	Mercury (Hg)	BLQ (0.05)	Not more than 1.0	ICP-MS
4.	Cadmium (Cd)	BLQ (0.05)	Not more than 0.3	ICP-MS

Table 4: Aflatoxin in Ointment.

S.No.	Aflatoxin (µg/kg)	Result (µg/kg)	Permissible Limit (µg/kg) as per API	Method of Testing
1.	Total Aflatoxin (B1+B2+G1+G2)	BLQ (1.0)	5.0 Max	LC-MS/MS
2.	Aflatoxin B1	BLQ (1.0)	2.0 Max	LC-MS/MS

Table 5: Pesticide residue in Ointment.

S.No.	Pesticide Residue	Result (mg/kg)	Requirement as per API (Max)
1.	Alachor	BLQ (0.01)	0.02
2.	Aldrin and Dieldrin (sum of)	BLQ (0.01)	0.05
3.	Azinphos-methyl	BLQ (0.01)	1.0
4.	Bromopropylate	BLQ (0.01)	3.0
5.	Chlordane (Sum of cis-, trans and Oxythlordane)	BLQ (0.01)	0.05
6.	Chlorfenvinphos	BLQ (0.01)	0.5
7.	Chlorpyriphos	BLQ (0.01)	0.2
8.	Chlorpyriphos-methyl	BLQ (0.01)	0.1
9.	Cypemethrin(and isomers)	BLQ (0.01)	1.0
10.	DDT (Sum of p,p-DDT, o,p-DDT, p,p-DDE and p,p-TDE)	BLQ (0.01)	1.0
11.	Deltamethrin	BLQ (0.01)	0.5
12.	Diazinon	BLQ (0.01)	0.5
13.	Dichlorvos	BLQ (0.01)	1.0
14.	Dithiocarbamates (as CS2)	BLQ (0.01)	2.0
15.	Endosulfan (sum of isomers and Endosulfan sulphate)	BLQ (0.01)	3.0
16.	Endrin	BLQ (0.01)	0.05
17.	Ethion	BLQ (0.01)	2.0
18.	Fenitrothion	BLQ (0.01)	0.5
19.	Fenvalerate	BLQ (0.01)	1.5
20.	Fonofos	BLQ (0.01)	0.05
21.	Heptachlor (sum of Heptachlor and Heptachlorepoxide)	BLQ (0.01)	0.05
22.	Hexachlorobenzene	BLQ (0.01)	0.1
23.	Hexachlorocyclohexane isomers (other than y)	BLQ (0.01)	0.3
24.	Lindane (y-Hexachlorocyclohexane)	BLQ (0.01)	0.6
25.	Malathion	BLQ (0.01)	1.0
26.	Methidathion	BLQ (0.01)	0.2
27.	Parathion	BLQ (0.01)	0.5
28.	Parathion-methyl	BLQ (0.01)	0.2
29.	Permethrin	BLQ (0.01)	1.0
30.	Phosalone	BLQ (0.01)	0.1
31.	Peperonyl butoxide	BLQ (0.01)	3.0
32.	Pirimiphos-methyl	BLQ (0.01)	4.0
33.	Pyrethrins (sum of)	BLQ (0.01)	3.0
34.	Quintozene (sum of Quintozene, Pentachloroaniline and methyl Pentachlorophenyl sulphate)	BLQ (0.01)	1.0

CONCLUSION

All four factors examined in the study are crucial for assessing drug safety and toxicity. The results revealed that the levels of microorganisms (fungus and bacteria) were below the allowable limits, indicating no potential for adverse effects. Heavy metals (arsenic, mercury, cadmium, and lead) were found to be below the limit of quantification. Aflatoxins B1, G1, B2, and G2, as well as pesticide residues, were absent in the test sample. The absence of these toxic substances in the test drug confirms that it is safe and free from significant toxic effects. Based on the findings of this study, all safety criteria tested on the Polyherbal Unani Ointment were found to be within the acceptable limits, indicating that the test drug is safe for use in preclinical and clinical studies.

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