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Safety Study of a Unani Pharmacopeial compound beneficent in Urticaria (*Shara*)

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Abstract : Safety is a vital component in the provision of herbal medicines and herbal products for health care, and an essential component of quality control. The quality of herbal medications has a great impact on their efficacy and safety. So, it is necessary to identify the risks associated with their use. One *nuskha* (prescription) was taken from Unani Pharmacopoeia consisting of single drugs Zizyphus sativa (Unnab), Prunus domestica (Aloo Bukhara), Coriandrum sativum (Kishneez Khushk), Fumaria officinalis (Shahtra) and was subjected to safety study as per the guidelines of WHO. All the ingredients of the test formulation were coarsely powdered separately in an electric grinder and then mixed. The powder was then investigated for Microbial load determination, Heavy metal determination, Aflatoxins estimation, Pesticidal residue estimation and Test for specific pathogens.The result demonstrated that heavy metals i.e., Arsenic, Mercury, Cadmium, and lead were found present within permissible limit. Total bacterial count and total yeast and mould count were many folds lower than the permissible limit i.e. 1220 and 120 cfu/gm respectively, the specific pathogens like *E. coli*, *Salmonella*, *S. aureus and P aeruginosa* were found to be absent whereas Aflatotoxins B1, G1, B2, G2 as well as Pesticide residue were found absent in the test drug. All the four examined parameters in the study are thought to be significant in determining the safety and toxicity of drugs. Since they were either present within permissible limit or were completely absent. Therefore, the test drug is considered to be safe and can safely be used for pharmacological and clinical purposes.

IndexTerms **- Safety study, Nuqu-e-Unnab, Urticaria, microbial load, heavy metal.**

INTRODUCTION

Chronic spontaneous urticaria (CSU) being a highly prevalent disease, has a great impact on the general population representing a burden for national health systems due to its variability and yet unknown pathogenesis (Marco, 2018). The lack of clinical and laboratory biomarkers able to define the severity and prognosis usually leads to frustration of patients as well as of physicians. Moreover, CSU has a variable rate of response to the approved newest therapies and only provide symptomatic relief as well as long term use of these drugs have been proved to cause many side effects. With these drawbacks, the treatment of CU is unsatisfactory in the conventional therapies. Due to this reason herbal medicines from Unani literature were explored for the treatment of this disease. One *nuskha* (prescription) was taken from Unani Pharmacopoeia consisting of single drugs Zizyphus sativa (Unnab), Prunus domestica (Aloo Bukhara), Coriandrum sativum (Kishneez Khushk) and Fumaria officinalis (Shahtra) (Arzani, 1998). These drugs possessed *musakkin* (sedative), *muaddil Dam wa Safra* and *musaffi Dam* (blood purifier) properties. However, prior to pharmacological and clinical trials, it was submitted to a safety study, as required by WHO regulations to assure the safety of a medicine.

Safety is a vital component in the provision of herbal medicines and herbal products for health care, and an essential component of quality control. The safety of herbal medicines has become major concern to national health authorities as well as the general public due to its rapid global expansion and popularity (WHO, 2004). The quality of herbal medications has a great impact on their efficacy and safety. So, it is

necessary to identify the risks associated with their use (Tripathy et al., 2015). Heavy metals, mycotoxins, pesticide residues, polycyclic aromatic hydrocarbons (PAHs), and fumigants are some of the factors that can affect the quality of these herbs. These contaminants might accumulate during the cultivation, storage, and processing of herbs and may have adverse health impacts on consumers. Exposure to these chemicals may have a cumulative, additive, or synergistic impact on human health. They can affect hormones in both men and women, leading to infertility, immunological suppression, carcinogenesis, and teratogenic consequences. Therefore, it is important that the Herbs and herbal products should be free from contaminants or at least be present within normal permissible limits so that it does not cause any detrimental effect on human health (De Smet et al., 1992).

According to the International Agency for Research on Cancer (IARC) Aflatoxin cause hepatocellular carcinoma. If the medicinal herbs and the plants are not properly dried or preserved after preparation, aflatoxin contamination might occur in the product. Also, Humans consume herbal products for extended periods of time; therefore, if they contain contaminants of aflatoxin, the exposure of the consumers to aflatoxin will be prolonged (Jeyaraj et.al, 2022).

The health of humans and animals may be at risk from heavy metal (like pd, cd, hg, cu, As) uptake by plants and subsequent deposits along the food chain. They are considered to have adverse impacts in human even at extremely low doses due to their poor renal elimination rates. They accumulate in soft tissues and are difficult for the body to digest. They interact with several normal biochemical and metabolic processes which results in adverse effects. Excessive dietary heavy metal intake has been connected to a number of health issues, such as weakened immunity, heart difficulties, foetal deformity, and altered neurological and psychosocial behaviour (Luo et al., 2020).

Pesticides are mostly sprayed on crops to protect them from a variety of pests. Pesticide residues have also been found in raw medicinal plant components such as such as HCH, Aldrin, heptachlor, chlorpyrifos, and malathion etc. Due to increasing demand and awareness of the medicinal plants, the commercial production of medicinal plants has also been increased resulting into excessive use of pesticides such as spraying, soil treatment, use of contaminated water source and cultivation in contaminated soil and use of fumigant while storing (WHO, 2007).

In the present study, the pharmacopoeial formulation was taken to evaluate its safety study as per the guidelines of WHO before subjecting to pharmacological and clinical studies.

MATERIALS AND METHODS

1. Drugs preparation

Before undertaking the safety study, the following processes were carried out to prepare the test drug:

- Collection and identification of ingredients
- Processing of the raw material

All the crude drugs were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, India, except *Aloo Bukhara* which was freshly procured from local market. It was washed, cleaned from undesirable substances, sliced and then dried in oven as well as in sunlight. All the other ingredients were also cleaned from earthy and undesirable substances and then dried in sunlight. The identification of the drugs was confirmed by Pharmacognosy section of Department of *Ilmul Advia*, Faculty of Unani Medicine, AMU, Aligarh on the basis of ethno-botanical description written in the literatures. For the purpose of documentation and future reference, the specimens of every single drug have been submitted to Ibn Baitar Museum of the Department of Ilmul Advia, Faculty of Unani Medicine, A.M.U. Aligarh bearing the voucher numbers are listed in Table 1.

Table 1: List of single drugs used in the Formulation

S.No.	Unani Name	Voucher number	Scientific name	Family	Parts used	Quantity (in gm)
1	***Unnab***	SC-404/24	*Zizyphus sativa*	Rhamnaceae	Fruit	21 gm
2	***Aloo Bukhara***	SC-406/24	*Prunus domestica*	Rosaceae	Fruit	164 gm
3	***Kishneez***	SC-403/24	*Coriandrum sativum*	Apiaceae	Fruit	7.5 gm
4	***Shahtra***	SC-405/24	*Fumaria officinalis*	Papaveraceae	Whole plant	9 gm

(Hakeem 1999; Khan 2012; Khan 2014; Khan 2018; Ibn Baitar, 1999).

All single ingredients of the test formulation were coarsely powdered separately in an electric grinder and then mixed in accordance with the proportion given in the table 1.

2. Safety parameters

The powder was investigated for Microbial load determination, Heavy metal determination, Aflatoxins estimation, Pesticidal residue estimation and Test for specific pathogens at Delhi Test House (DTH), A-62/3, G.T Karnal road, Azadpur, Delhi, India vide no. QR0302 Report no. 2350220910IM126015 Letter Date 07/09/2022.

2.1 Heavy Metals Analysis

The content of metallic impurities i.e. heavy metals including Arsenic, Mercury, Cadmium, and lead were determined in the test sample using Atomic Absorption Spectroscopy (AAS) (Table-2).

2.2 Microbial Load Determination

2.2.1 Total viable aerobic count

The total viable aerobic count (TVC) of the test drug was carried out for identification of anti-bacterial activity as stated in the test procedure, using plate count results.

2.2.2 Pretreatment of the test drug

The test sample was pre-treated by dissolving it using a suitable technique, and any antibacterial properties were eliminated by dilution or neutralization depending on the nature of the test drug. The test drug sample was diluted using MM1275-500G from Hi-media Labs in Mumbai, India, which is a buffered sodium chloride-peptide solution having pH 7.0.

2.2.3 Plate count for bacteria

One ml of the pre-treated test sample and around fifteen ml of the liquefied casein-soybean digest agar were combined in a 9-10 cm diameter petri dish at a temperature lesser than forty-five degrees Celsius. As an alternative, the test sample was dispersed throughout the solidified medium's surface. The same dilution was used to make two plates, which were then inverted and incubated at 30-35 °C for 5 days, unless a more precise count was obtained in a short period of time. The number of colonies that subsequently developed was counted, and the plates with the highest number of colonies but up to a maximum of 300, were used to determine the results (CCRUM, 2009).

2.2.4 Plate count for fungi

One ml of the pre-treated test sample and around fifteen ml of the liquefied Sabouraud dextrose agar were combined in a 9-10 cm diameter petri dish at a temperature lesser than forty-five degrees Celsius. As an alternative, the test sample was dispersed throughout the solidified medium's surface. The same dilution was used to make two plates, which were then inverted and incubated at 20-25 °C for 5 days, unless a more precise count was obtained in a short period of time. The number of colonies that subsequently developed was counted, and the plates with the highest number of colonies but up to a maximum of 100, were used to determine the results (CCRUM, 2009).

2.3 Estimation of Aflatoxins

Using LC-MS/MS, aflatoxins B1, G1, B2, and G2 were determined in the test sample of the specified herbal medicine powder. The 60% acetonitrile/water (20 ml) and the test drug sample of 2 grams were rapidly blended at high speed for two minutes. The mixed sample was then centrifuged at 1600 rpm for 10 minutes. The supernatant was collected and diluted with 2 ml filtrate in 48 ml of Phosphate Buffered Saline (PBS, pH 7.4) to obtain a solvent concentration equals to or less than 2.5%. Similarly, methanol/water was prepared by combining 2 ml of test sample with 14 ml of Phosphate Buffered Saline (PBS, pH 7.4) to obtain a solvent concentration equals to or less than 10%. The sample diluent was run through the immunoaffinity column with the speed of 5 ml/min. Later on, the column was washed with 20 ml of distilled water at a flow rate of around 5 ml/min, then quickly dried by passing air through it. 1.5 millilitres of distilled water was mixed with the sample elute. A 500 µl sample volume was introduced into the LCMS-MS. Sample peak heights or areas were compared to the total aflatoxin standard to determine the aflatoxin concentration in the sample (Ventura et. al., 2004)

2.4 Estimation of Pesticide Residue

The test for the assessment of specific pesticide residues like Organo-chloride, Organo-phosphorus and Pyrethroids compounds were conducted using GC-MS/MS. (Table-6).

RESULT

Result of the safety study revealed that the presence of heavy metals i.e., Arsenic, Mercury, Cadmium, and lead were found present within permissible limits (Table 2). The total bacterial count and total yeast and mould count were many folds lower than the permissible limit i.e. 1220 and 120 cfu/gm respectively (Table 3). Specific pathogens like *E. coli, Salmonella, S. aureus and P aeruginosa* were found to be absent in the test drug (table 4). Aflatotoxins B1, G1, B2, G2 as well as Pesticide residue were absent in the test drug (table 5 and 6 respectively).

Table 2: Heavy metals in test drugs

S.No.	Test Parameters	Result (Mg/Kg)	Permissible Limit (Mg/Kg)
1.	**Lead (Pb)**	Not detected (2.5)	NMT 10
2.	**Mercury (Hg)**	Not detected (0.5)	NMT 1
3.	**Arsenic (As)**	Not detected (1.25)	NMT 3
4.	**Cadmium (Cd)**	Not detected (0.25)	NMT 0.3

*NMT- not more than

Table 3: Microbial load in the test drug

S.No.	Test Parameters	Result (cfu/gm)	Permissible Limit (cfu/gm)
1.	**Total bacterial count**	1220	NMT 10^5
2.	**yeast and mould count**	120	NMT 10^3

Table 4: Test for specific pathogens

S.No.	Pathogens	Result	Permissible Limits as per API
1.	**E. coli**	Absent	Absent
2.	**Salmonella**	Absent	Absent
3.	**S. aureus**	Absent	Absent
4.	**P. aeroginosa**	Absent	Absent

Table 5: Aflatoxins in the test drug

S.No.	Aflatoxins	Result (Mg/Kg)	Limit of quantification (mg/kg)	Permissible Limit (Mg/Kg)
1.	**Aflatoxin B_1**	Not Detected	0.001	NMT 0.5
2.	**Aflatoxin G_1**	Not Detected	0.001	NMT 0.5
3.	**Aflatoxin B_2**	Not Detected	0.001	NMT 0.1
4.	**Aflatoxin G_2**	Not Detected	0.001	NMT 0.1

*NMT- not more than

Table 6: Pesticide residue in the test drug

S.No.	Pesticide	Result (Mg/Kg)	Limit of quantification (mg/kg)	Permissible Limit (mg/kg)
1.	**Alachlor**	Not Detected	0.02	0.02
2.	**Aldrin & Dieldrin (Sum of)**	Not Detected	0.04	0.05
3.	**Azinophos-methyl**	Not Detected	0.04	1.0
4.	**Bromopropylate**	Not Detected	0.08	3.0
5.	**Chlordane (Sum of cis, trans and oxychlordane)**	Not Detected	0.04	0.05
6.	**Chlorfenvinphos**	Not Detected	0.04	0.5
7.	**Chlorpyrifos**	Not Detected	0.04	0.2
8.	**Chlorpyrifos-methyl**	Not Detected	0.04	0.1
9.	**Cypermethrin (and isomers)**	Not Detected	0.10	1.0
10.	**DDT (Sum of pp-DDT, pp-DDE and pp-TDE)**	Not Detected	0.04	1.0
11.	**Deltamethrin**	Not Detected	0.10	0.5
12.	**Diazinon**	Not Detected	0.04	0.5
13.	**Dichlorvos**	Not Detected	0.04	1.0
14.	**Dithiocarbamates (as CS2)**	Not Detected	0.10	2.0
15.	**Endosulfan (Sum of Isomer & Endosulfan sulphate**	Not Detected	0.04	3.0
16.	**Endrin**	Not Detected	0.04	0.05
17.	**Ethion**	Not Detected	0.04	2.0
18.	**Fenitrothion**	Not Detected	0.04	0.05
19.	**Fenvalerate**	Not Detected	0.1	1.5
20.	**Fonofos**	Not Detected	0.04	0.05

21.	**Heptachlor (Sum of Heptachlor & Heptachlor epoxide)**	Not Detected	0.04	0.05
22.	**Hexachlorobenzene**	Not Detected	0.04	0.1
23.	**Hexachlorocyclohexane isomer (other than y)**	Not Detected	0.04	0.3
24.	**Lindane (y-Hexachlorocylohexane)**	Not Detected	0.04	0.6
25.	**Malathion**	Not Detected	0.04	1.0
26.	**Methidathion**	Not Detected	0.04	0.2
27.	**Parathion**	Not Detected	0.04	0.5
28.	**Parathion Methyl**	Not Detected	0.04	0.2
29.	**Permethrin**	Not Detected	0.04	1.0
30.	**Phosalone**	Not Detected	0.04	0.1
31.	**Piperonyl butoxide**	Not Detected	0.04	3.0
32.	**Primiphos Methyl**	Not Detected	0.04	4.0
33.	**Pyrethrins (sum of isomer)**	Not Detected	0.1	3.0
34.	**Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)**	Not Detected	0.1	1.0

*DDT- Dichloro diphenyl trichloroethane
*DDE- Dichloro diphenyl dichloroethylene
*TDE- 2,2-thiodiethanol

DISCUSSION

Herbal drugs are often polluted with different types of toxicants, which in turn cause deterioration in their quality and variation in their chemical composition. A change in the qualitative or quantitative attributes of a drug may produce undesired effect. Therefore, it is necessary to ensure the safety of a drug before any clinical trial. The herbal drugs contaminated with various microorganisms, heavy metals (lead, cadmium, arsenic and mercury), pesticides and aflatoxins, can modify the pharmacological activity and occasionally cause serious side effects. Therefore, it has now been made mandatory by WHO to perform their safety profile.

The heavy metals transferred from contaminated soil to plants and then to humans tend to accumulate in the body. These are excreted at a very low rate and can lead to induce several chronic and acute diseases. The concentration of heavy metals in the test drugs was found within the normal limits indicating that the test drug is free from heavy metal contamination (Table 2).

Sometimes the herbal drugs are intoxicated by **microbes** present in them. These microbial toxins make the drugs unfit for humans as they may develop a new disease or worsen the existing one rather than curing it. Considerable interest, therefore, lies in an investigation on microbial contamination associated with drug samples. The test drug was tested for specific pathogens i.e., *E. coli*, *Salmonella*, *S. aureus and P. aeruginosa*, which were found to be absent in the test drug (Table 3 and 4).

Test for **aflatoxins** was carried out to detect the presence of aflatoxins, B_1, G_1, B_2 and G_2. Many countries have set the maximum limits for different types of aflatoxins as these are highly toxic contaminants in any

material of plant origin and may cause very serious side effects. Screening of test drug for aflatoxins showed that the test drug does not have aflatoxins. (Table 5).

The use of **pesticides** in agricultural practices during cultivation and storage, to protect the crops against insects, weeds, fungi and other pests, leads to the exposure of herbal drugs to various harmful components i.e., chlorine and phosphorus. These pesticides can have both acute and chronic side effects; therefore, they are checked for their limits. The findings suggested that the pesticidal residues in the test drug were within the normal limits (Table 6). The findings demonstrated that the test drug qualifies the safety criteria as recommended by WHO. Since it is free from the major toxic contaminants, therefore, may be used in the patients safely.

CONCLUSION

All the four examined parameters in the study are thought to be significant in determining the safety and toxicity of drugs. This study demonstrated that the microbial count in the test drug was within permissible limits and it does not contain any specific pathogens i.e., *E. coli*, *Salmonella, S. aureus and P. aeruginosa. Heavy metals* and pesticide residue were found within permissible limits. Aflatoxins were not detected in the study. Hence, the test drug is considered safe to be used for pharmacological and clinical purposes.

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