Safety Study of a Single Unani Drug Khare-e-khasak Khurd (*Tribulus terrestris* Linn.)

[1]Mohd. Waseem, [2]Abdul Latif, [3]Sumbul Rehman, [1]Reesha Ahmed and [1]Zafar Javed Khan

Department of Ilmul Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh-202002

Abstract

Present study was aimed to evaluate safety parameters in Khar-e-Khasak Khurd (*Tribulus terrestris* L.) - a very common drug used in Unani Medicine for its lithotriptic and aphrodisiac effect used in urinary disorders and impotence. Study reveals the presence of heavy metals lead, cadmium, mercury and arsenic within permissible limit as per WHO guidelines while aflatoxins, pesticides and microbial load was found to be absent in the crude drug sample. It can be said that the drug is free from toxicity.

Keywords: Khar-e-khasak khurd (*Tribulus terrestris* L.), Safety study, Herbal Medicine, WHO Guidelines

Introduction

Tribulus terrestris Linn (Family-Zygophyllaceae) known as Khar-e-khasak in Unani Medicine, Gokhru in Urdu, is a thorny fruit of *T. terrestris* mentioned in many classical Unani literature (Ghani, ynm). It has been used in India and China since time immemorial for health ailments as lithotripter, aphrodisiac and useful in strangury (Hashim *et al*., 2014). *T. terrestris* is an annual or perennial plant growing throughout India and other warm countries such as Ceylon (Chopra, 1958). According to Unani literature, Khar-e-khasak has been described morphologically of two varieties small (khurd) and Kalan (large/big) according to the size of fruit; among which mostly Khurd variety is medicinally used (Kabeer uddin, y.n.m). Renowned Unani scholars Rhazes (865-925 AD) mentioned Khar-e-khasak as lithotriptic, aphrodisiac, demulcent, and useful in strangury in his book *Kitabul Mansoori* (Razi, 2008), Usr-al-Baul (Dysuria), Sozak (Gonorrhoea), Urinary disorders, incontinence of urine and impotence (Khory, 1985), useful in strangury, vesicular calculi, pruritus ani, alleviate burning sensation (Kiritikar and Basu,1996). It possesses many actions like Mudir-i-Baul (Diuretic) (Chopra, 1958; Nadkarni, 1954), Musaffie dam (Blood purifier), have cooling effect and tonic to the body and used in calculus affection, kidney diseases and painful micturition (Dey1980).

Current practices of harvesting, production, transportation and storage of herbal drugs cause additional contamination and microbial growth proliferation of microorganism that may result from failure to control the moisture levels of herbal medicines during transportation and storage (Anonymous, 2007). Aflatoxin B_1, G_1, B_2, G_2, are fungal secondary toxic metabolites produced by *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius*. Aflatoxins are the strongest natural carcinogens and their main target organ is the liver. The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 in the group 1 as a human

[3*] Author for correspondence;

carcinogens and aflatoxin G_1, B_2 and G_2 in the group B_2 as possible carcinogens to humans (Meritxell Ventura, 2004). Contamination of herbal materials with toxic substances such as arsenic can be attributed to many factors. These include environmental pollution (i.e. contaminated emissions from factories, leaded petrol, and contaminated water including runoff water which finds its way into rivers, lakes and sea, and some pesticides), Soil composition and fertilizers. The contamination of the herbal material leads to contamination of the products during various stages of the manufacturing process (Anonymous, 2007). The worldwide consumption of herbal medicines is enormous, so in terms of population exposure alone, it is essential to identify the risks associated with their use as safety of herbal medicines is an important public health issue (Anonymous, 2004). Present study is an attempt to assess these safety parameters in a well known herbal drug used in Unani Medicine Khar-e-khasak khurd (*T. terrestris* Linn.).

Material and Methods

Sample preparation:

The test drug Khare-e-khasak khurd (*T. terriestris* Linn) was procured from local market of Aligarh city in the month of May 2016 and was properly identified according to the morphological features mentioned in botanical and Unani literature & then further confirmed in Pharmacognosy section of department of Ilmul Advia, A.M.U., Aligarh. A herbarium sample of the test drug was prepared & submitted to Mawalid-e-salasa museum of the department after identification for further reference with Voucher no, SC-0188/15.

The drug was cleaned from the earthy material, washed with double distilled water and dried at 45º C in hot air oven to powder it in electrical grinder. There after the drug was passed through sieve no. 80 to confirm its fineness and uniformity of particle size. Finally the powdered drug was stored in an air tight container for experimental study.

The powder of test drug was studied to evaluate the presence of microbial load, pesticides residue, aflatoxins and heavy metals at Delhi Test House, Azadpur (New Delhi) as per WHO Guidelines.

1. Microbiological determination tests

Total viable aerobic count (TVC)

For detection of the anti-bacterial activity of the test drug, the total viable aerobic count (TVC) of the test drug was carried out, as specified in the test procedure, using plate count, results are shown in table-1.

Pre-treatment of the test drug

Depending on the nature of the herbal drug sample used, it was dissolved using a suitable method and any antimicrobial property present in the sample was eliminated by dilution or neutralization. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G, Himedia Labs, Mumbai, India) was used to dilute the test sample.

Test procedures

Plate count for bacteria and fungi

For bacteria: 1 ml of the pretreated test sample was added to about 15 ml of the liquefied casein-soybean digest agar in a petridish of 90 mm diameter at a temperature not exceeding 45 0C. Alternatively the test sample was spreadon the surface of the solidified medium. Two dishes were prepared with the same dilution, they were inverted and incubated at 30-350C for 48-72 hrs. unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with the largest number of colonies, up to a maximum of 300.

For fungi: 1 ml of the pretreated test sample was added to about 15 ml of the liquefied Sabouraud glucose agar with antibiotics in a petridish of 90 mm diameter at a temperature not exceeding 450C. Alternatively the test sample was spreadon the surface of the solidified medium. Two dishes were prepared with the same dilution; they were inverted and incubated at 20 - 250C for 5 days, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with not more than 100 colonies. (Lohar, 2007).

2. Estimation of Aflatoxins sample preparation

The test for determination of the aflatoxins was carried out using LCMS-MS. 2gm of test drug was blended at high speed with 20 ml of 60% acetonitrile/water for two minutes. The blended sample was centrifuged for ten minutes using 1600 rpm (av.), supernatant was retained and diluted with 2 ml of filtrate with 48 ml of phosphate buffered saline (PBS, pH 7.4) to give a solvent concentration of 2.5% or less; methanol/water was prepared by taking 2 ml of sample and diluted with 14 ml of PBS (pH 7.4) to give a solvent concentration of 10% or less. The sample diluent was passed through the immunoaffinity column at a flow rate of 5 ml/ min. The column was then washed by passing 20 ml of distilled water through the column at the flow rate of approximately 5 ml/ min and dried by rapidly passing air through the column. 1.5 ml of distilled water was added to the sample elute.

500 µl of sample was injected onto the LCMS-MS (LC- Perkin, MS Applied Bio System, Model No.2000, Mobile Phase). A- Water 100%, B-ACN 100%, Column oven temperature = 30, Column ZORBAX Rx c18, narrow base 2.1×150 mm - 5 micron, Flow = 0.750 ml). The aflatoxin concentration was quantified by comparing sample peak heights or areas to the total aflatoxin standard (R-Biopharm) (Lohar, 2007). Results so found are shown in table-2.

3. Heavy metals

Heavy metals including Arsenic, Mercury, Cadmium and lead were determined in the test sample using Atomic Absorption Spectroscopy (table-3).

4. Estimation of pesticidal residue

The test for determination of the aflatoxins was carried out using GC/MS. The test was done for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorous compounds, and Pyrethroids compound (Ramkrishanan, *et al*, 2015) as depicted in Table-4.

Figure 1: Khar-e-khasak Khurd (*Tribulus terrestris* Linn.)

Plant of *Tribulus terrestris* Linn.

Fruits of *Tribulus terrestris* Linn.

Table 1(a): Microbial load in Khar-e-khasak khurd

S. No.	Microbes	Result	Permissible limit
1.	Total Bacterial Count	680	Not more than $1x10^5$ cfu/gm
2.	Total Yeast & Mould	50	Not more than 1 $x10^3$ cfu/gm

Table 1(b): Test for specific pathogens in Khar-e-khasak khurd

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

Table 2: Aflatoxin in Khar-e-khasak khurd

S. No.	Aflatoxin	Result	LOQ (mg/kg)	Permissible Limit (mg/kg)
1	Aflatoxin B_1	BLQ	0.001	Not more than 0.5
2.	Aflatoxin G_1	BLQ	0.001	Not more than 0.5
3.	Aflatoxin G_2	BLQ	0.001	Not more than 0.1
4.	Aflatoxin B_2	BLQ	0.001	Not more than 0.1

LOQ = Limit of quantification

BLQ = Below the limit of quantification

Table 3: Heavy Metal in Khar-e-khasak khurd

S. No.	Test parameter	Result (mg/kg)	LOQ (mg/kg)	Permissible limit (mg/kg)
1.	Lead (Pb)	Not detected	2.50	Not more than 10
2.	Mercury (Hg)	Not detected	0.5	Not more than 1
3.	Arsenic (As)	Not detected	1.25	Not more than 3
4.	Cadmium (Cd)	Not detected	0.25	Not more than 0.3

LOQ = Limit of Quantification

BLQ = Below the limit of Quantification

Table 4: Pesticidal residue in Khar-e-khasak khurd

S. No.	Pesticide	Result	LOQ (mg/kg)	Permissible Limit (mg/kg)
1.	Alachlor	Not detected	0.02	0.02
2.	Aldrin & Dieldrin	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	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	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	Not detected	0.01	2.0
15.	Endosulfan (Sum of Isomer and Endosulfan sulphate)	Not detected	0.04	3.0

S. No.	Pesticide	Result	LOQ (mg/kg)	Permissible Limit (mg/kg)
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	0.10	1.5
20.	Fonofos	Not detected	0.04	0.05
21.	Heptachlor (Sum of Heptacholar & Heptachlor epoxide)	Not detected	0.04	0.05
22.	Hexachlorobenzene	Not detected	0.04	0.1
23.	Hexachlorocyclohexane isomer (other than ý)	Not detected	0.04	0.3
24.	Lindane (ý-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	Not detected	0.10	3.0
34.	Quintozen (Sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	Not detected	0.10	1.0

Results and Discussion

All four parameters undertaken in the study are considered instrumental to determine the safety/ toxicity of drugs. The result of the study demonstrated that heavy metals (Arsenic, Mercury, Cadmium and Lead) were not found to be present. Their presence cause serious effects on human body as Aflatoxin (B_1, B_2, G_1 and G_2) cause serious side effects such as hepatotoxity, carcinogenetic etc. Microbial count (Bacterial, yeast and Mould) were found below permissible limit, which is unable to produce any toxicity. This drug is also free from pesticide residue contamination.

The study revealed that the safety parameters carried out on Khar-e-khasak khurd (*Tribulus terrestris*) are within the permissible limits which indicate that drug is quite safe as per WHO requirement.

References

Anonymous, 2004. WHO guidelines on safety monitoring of herbal medicine in pharmacovigilance system. WHO, Geneva.

Anonymous, 2007. WHO guidelines for assessing quality of herbal medicine with reference to contaminants and residues, World Health Organization Geneva, pp.14-15.

Chopra, R.N., Chopra, K.C and Kapoor, L. D., 1958. Indigenous Drugs of India, 2nd Edition, U. N. Dhur and Sons Private Limited, 15, Bankim Chatterjee Street Calcutta-12, p. 430

Dey.C.A., 1980. "Indian Medicinal Plants used in Ayurvedic Preparation. Bishen Singh Mahendra Pal Singh, Dehradun, p. 52.

Ghani, N., YNM. K hazain al-advia, New Delhi, Idara Kitab us shifa.

Hashim, *et al*., 2014. Medicinal properties Phytochemistry and Pharmacology of *Tribulus terrestris* L (Zygophyllaceae). *Pak J botany*, 46:399-404

Kabeeruddin, YNM. Makhzanul mufridat, New Delhi, Ejaz publishing house.

Khory, R.N and Katrak, N.N., 1985. Materia Medica of India and their Therapeutic. Neeraj Publishing House Delhi. p. 148.

Kirtikar, K. R., Basu, B. D., 1996. Indian Medicinal Plants, IInd Ed. International Book Distributers, Rajpur road, Dehradun, India. Vol. IV, p. 420

Lohar, D.R., 2007. Protocol for testing of Ayurvedic, Siddha & Unani Medicine pharmacopoeial Laboratory for Indian Medicines, Ghaziabad, pp. 77-89.

Meritxell Ventura, Antonio, G. Anaya, I., Diaz, J., Broto, F., Agut, M., Comellas L. 2004. Determination of Aflatoxin B1, G1, B2, G2, in medicinal herbs by liquid chromatography- tandem mass spectrometry, *Journal of Chromatography* A, 1048(2): 25-29.

Moses, A.G. Maobe, Erastus Gatebe, Leonard Gitu and Henry Rotich, 2012. Profile of Heavy Metals in Selected Medicinal Plants used for the treatment of Diabetes, Malaria and Pneumonia in Kisii region, Southwest Kenya. *Global Journal of Pharmacology* 6(3): 245-251.

Nadkarni, A.K., 1954. Indian Materia Medica, Edi.3rd Popular Book Depot, Bombay, Dhootapapeshwar Prakashan Ltd. Panvel, Vol.I, p.1229.

Ramkrishanan, G. Gayathri V. Sathia, S. Parameswari, R.P. Saravana, C.B., 2015. Physicochemical and phytochemical standardization of Thraatchathi chooranam- A polyherbal formulation. *Journal of Pharmaceutical Science & Research* 7(6):305-313.

Razi, A.M.Z., 2008. Kiabal hawi, Vol.22, New Delhi, CCRUM, 181