Journal of Advanced Research in BioChemistry and Pharmacology
Volume 1, Issue 1&2 - 2018, Pg. No. 33-37
Peer Reviewed & Open Access Journal

Research Article

Safety Study of a Drug Tukhm-e-Kharpaza (*Cucumis Melo* Linn.)

Mohd Waseem[1], A. Rauf[2], Sumbul Rehman[3], Reesha Ahmed[4]

[1]Department of Psychiatry, All India Institute of Medical Sciences, New Delhi.
[2,3,4]Department of Ilmul Advia, A.K.T.C., A.M.U., Aligarh.

Abstract

Drugs used in traditional medicines are liable to be contaminated with toxic substances. Plants are prone to be contaminated with agricultural practices and lead to depression, Memory loss, Loss of sensation, chronic renal failure etc. Safety study of herbal drugs and food items is now mandatory as per WHO guidelines. A critical evaluation of their safety is therefore important. One particular safety issue relates to the possibility of contamination of herbal medicinal products. Herbal medicinal products may be toxic due to adulterer with synthetic drugs or contaminated with harmful ingredients ranging from pesticides to heavy metals. Lead was the most commonly found metal, followed by mercury and arsenic.

All the safety parameters were found below the permissible limit as per WHO guidelines, its shows that the drug Tukhm-e-Kharpaza *(*Linn.*)* is free from toxicity.

Keywords: Tukhm-e-Kharpaza, Safety study, Heavy metal, Pesticide, *Cucumis melo*

Introduction

In Unani System of Medicine, there are large number of single and compound drugs which have antibacterial and antifungal activity and used in many infectious diseases. Tukhm-e-Kharpaza is one of the important single drug which is mention in many classical books. Tukhm-e-Kharpaza is also called Kharbuzah in urdu, seeds of *Cucumis melo* Linn. of family Cucurbitaceae. *Cucumis melo* Linn. is a well-known plan indigenous and largely cultivated in India, particularly in hot, dry, North-Western areas, Northern Bengal, Kashmir and Afghanistan[1,2,3] . It is creeper having rough surface, leaves are round in shape, flowers are of different shapes and colours, plant commonly found in India and Pakistan[4]. It possess many Pharmacological actions like Diuretic *(Mudirr-e-Baul)*[4,5,6]; Lithotriptic (*Mufattit-e-Hasat*)[4,7]; Demulcent (*Mulattif)*[8,9]; Tonic (*Muqawwi)*[3,9,1;] Emmenagogue *(Mudirr-e-Haiz)*[4,7,11] and used in Burning Micturition *(Hirqat-ul-Baul)*[5,6,7,9,11,12,13,14]; Urinary infection *(Tadiya Majariy-e-Baul)*[3]; Kidney and Bladder stone *(Sang Gurda wa Masana)*[5,7,14]; Anuria (*Ehtebas-e-Baul,* Amenorrhoea (*Ehtebas-e-Haiz)*[5,7]; Strangury *(Taqteer-ul-Baul)*[4,9] and many other diseases.

Drug materials normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. Current practices of harvesting, production, transportation and storage may cause additional contamination and microbial growth proliferation of microorganism may result from failure to control the moisture levels of herbal medicines during transportation and storage[15]. 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 carcinogens and aflatoxin $G_{1,}$ B_2 and G_2 in the group B_2 as possible carcinogens to humans[16]. Contamination of herbal materials with toxic substances such as arsenic can be attributed to many factors. These include environmental pollution (i.e. contaminated

Corresponding Author: Mohd Waseem. Department of Psychiatry, All India Institute of Medical Sciences, New Delhi.
E-mail Id: wasmsss@gmail.com
How to cite this article: Waseem M, Rauf A, Rehman S et al. Safety Study of a Drug Tukhm-e-Kharpaza (*Cucumis Melo* Linn.). *J Adv Res BioChem Pharma* 2018; 1(1&2): 33-37.

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[15]. 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 healt issue [19].

Material and Methods

Sample preparation

The test drug Tukhm-e-Kharpaza (*Cucumis melo* Linn.) were procured from local market of Aligarh and properly identified according to the botanical and Unani literature, confirmed in pharmacognosy section of department of Ilmul Advia, Ajmal khan Tibbiya college, A.M.U. Aligarh. A herbarium sample of the test drug were prepared and submitted to Mawalid-e-salasa museum of the department after identification for further reference- Voucher no. SC-0186/15. The drugs were powdered in electrical grinder and there after the drug was passed through the sieve no. 80 to confirm its fineness and uniformity of particle size. Finally the powder was stored in 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, Delhi-110033.

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.

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 ^{0}C. 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 30-35^{0}C 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 45^{0}C. 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 - 25^{0}C 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[17].

Pesticidal residue

The test for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorous compounds, and Pyrethroids compound were conducted using GC/MS-Ms[18].

Aflatoxins

The test for determination of the aflatoxins was carried out using LCMS-Ms.

Heavy metals

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

Table 1.Heavy Metal in Tukhm-e-Kharpaza-

S.No.	Test parameter	Result (mg/kg)	LOQ(mg/kg)	Permissible limit (mg/kg)
1.	Lead (Pb)	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 2.Microbial load in Tukhm-e-Kharpaza-

S.No.	Microbes	Result	Permissible Limit
1.	Total Bacterial Count	620	Not more than $1x10^5$ cfu/gm
2.	Total Yeast & Mould	60	Not more than $1 x10^3$ cfu/gm

Table 3.Aflatoxin in Tukhm-e-Kharpaza-

S.No.	Aflatoxin	Result	LOQ	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 4.Pesticidal residue in Tukhm-e-Kharpaza

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
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 (Sumof Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	Not Detected	0.10	1.0

Table 5.Test for Specific Pathogens in Tukhm-e-Kharpaza

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

Discussion and Conclusion

All four parameters undertaken in the study are considered instrumental to determine the safety/ toxicity of a drugs. The result of the study demonstrated that heavy metals (Arsenic, Mercury, Cadmium and Lead) were not found to be present. Its presence cause serious effects on human body. As Aflatoxin (B1, G1, B2, G2) were also 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 drugs is also free from Pesticide residue contamination.

The results of the study revealed that the safety parameters carried out on Tukhm-e-Kharpaza (*Cucumis melo* Linn.) are within the permissible limits which indicate that it is quite safe as per WHO requirement.

Conflict of Interest: None

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Date of Submission: 2018-05-25
Date of Acceptance: 2018-06-14