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Safety study of a single Unani drug: Saad Kufi (*Cyperus scariosus*)

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Abstract
A World Health Organisation survey indicated that about 70-80% of the world poplutions rely on non-conventional medicine mainly of herbal sources in their primary healthcare. Recent study was aimed to evaluate safety parameters of Saad Kufi (*Cyperus scariosus*) a very common drug used in Unani System of Medicine for its cardiotonic and exhilarant effect. Safety study of herbal drugs and food items is now mandatory as per World Health Organization (WHO) guidelines. It includes determination of aflatoxin, heavy metals, Pesticide residue and microbial load. 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: Saad Kufi (*Cyperus scariosus*), safety study, herbal medicine, WHO guidelines

1. Introduction
Herbal drugs are the most widely used form in the management of diseases all over the globe, due in part to the widespread assumption that "natural' implies "harmless'. However, with their popularity and global market expansion, the safety of herbal products has become a major concern in public health [1]. Contamination of crude medicinal plants as well as their products has increasingly been reported. Many contaminants occur naturally in the ground and the atmosphere, such as radionuclides and metals. Some arise from past or present use of agents such as factory emmisions or persistent chemical residues. 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 [2]. Aflatoxin B1, G1, B2, G2, 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 Researchon Cancer (IARC) has classified aflatoxin B1 in the group1as a human carcinogens and aflatoxin G1, B2 and G2 in the group B2 as possible carcinogens to humans [3]. 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 run off 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[4].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 [5].

Present study is an attempt to assess these safety parameters in a well-knownherbal drug used in Unani System of Medicine Saad Kufi (*Cyperus scariosus*).

Cyperus scariosus is a hardy grass like perennial plant consisting of 600 species distributed in tropical and warm temperate region of the world. *Cyperus* is a greek word meaning sedge [6]. Medicinally the root of *Cyperus scariosus* is used for the same purpose as those of *Cyperus* rotundus [7, 8] and this have long been in use in Hindu medicine and perfumery under the Sanskrit name Nagar mustaka [8]. Of the essential character of the genus, it has been said, "Glumes chaffy, imbricate in two rows; *corol*. none;seed one, naked," [9]. Ibn Sina, the most significant thinkers and writers of Islamic golden age first time systematized the individual cardiac drugs in "Risala Advia Qalbia", which deals with 63 cardiac drugs which are claimed to be beneficial for heart ailments as well as for psychiatric ailments. He described Saad as a root of a plant which is nodular, long, slender and plants look a wheat plant [10, 11, 12]. Roots are thick, elongated, slender, black in colour, aromatic smell with pungent taste [11, 12].

Stem is about one hand long and prostrate surrounded by small leaves and nodes [11]. Leafless or leafy shoots are produced above ground. Infloresence is umbel or head like. Spikelets are one to many flowered [6] and are linear straw coloured [13] Best Saad is one which is solid/hard, thick, not easily fractured and have strong aroma [10, 11]. Ibn Sina named it as Sor Kufi. Inferior to this, is of reddish colour and inner surface is white and have aroma [12]. In Unani literature actions described are Exhilarant [12], cardiotonic [14], Stimulant [15] Nervine Tonic [14, 16, 17, 18] Deobstruent [10, 11, 12] Astringent, Dessicant [19] Carminative [11, 14, 17] Appetizer [18, 20], Anti-emetic [11, 17], Aphrodisiac [12, 20]. It strengthens the urinary bladder [11, 12], Mouth Freshner [11, 15, 18, 21], Hair Removar [11] Complexion enhancer [11, 12, 17, 18]. It is used in the Palpitation [12, 16, 20] weakness of heart, brain and stomach [17] General weakness [14] Chronic ulcer, Ascites [11] Chronic fever [11, 12, 16] Anti-nociceptive activity of methanol extract of *C. scariosus* leaves was examined by Alam *et al*. [23] Ramesh *et al*. (2012) examined that n-hexane extract of *C. scariosus* oil exhibited antidepressant activity in mice [24]. Chawda *et al*. (2014) studied the lipid-lowering and antioxidant activities of a hydroalcoholic extract of *Cyperus scariosus* Linn. Root (HCS) on guinea pigs fed with a high cholesterol diet [25].

The tubers yield 0.5 percent of an essential oil; an additional (0.5%) oil has also been obtained by acid hydrolysis of non - volatile portion. Several volatile compounds including cyperine (15.8%), isopatchoul-4(5)-en-3-one (16.5%), I-oxo-selina-4(14), 7(11)-diene, Selina -4(5), 7(11)-dien-12-ol and patchoulanol have been reported from the oil [19].

2. Material and Methods

2.1 Sample preparation: The test drug Saad Kufi (*Cyperus scariosus*) was procured from local market of Aligarh city in the month of April 2017 and was properly identified according to the morphological features mentioned in botanical and Unani literature, confirmed in Pharmacognosy section of department of Ilmul Advia, A.M.U., Aligarh. It was also authenticated by National Institute of Science Communication and Information Resources, New Delhi (NISCAIR /RHMD/ Consult/ 2017/ 3064-13-1). The sample of the test drug was submitted to Mawalid-e-Salasa Museum of the Department after identification, for future reference with the voucher number of Voucher No. SC-0220/17.

The drug was cleaned from the earthy material, washed with double distilled water and dried at 45ºC in hot air oven and 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 sstudy.

The powder of root of Saad Kufi (*Cyperus scariosus*) was studied to evaluate the presence of microbial load, pesticides residue, aflatoxins and heavy metals at Delhi Test House, Azadpur, Delhi-110033 [QR-0302 Report No 25531709251M-81010 Sample Dated 25/09/2017 Reported on 03/10/2017].

2.2. Microbiological determination tests

2.2.1 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.

2.2.2 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.

2.2.3 Plate count for bacteria

1 ml of the pre-treated 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.

2.2.4 Plate count for fungi

1 ml of the pre-treated 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 [26].

2.3 Heavy metals

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

2.4 Estimation of Aflatoxins Sample preparation

The test for determination of the aflatoxins B_1, B_2, G_1, and $G_2$was carried out using LC-MS/MS.2gm of test drug was blended at high speed with 20 ml of 60% acetonitrile/water for twominutes. 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) [26].

2.5. Pesticide residue

The test for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorus compounds and Pyrethroids compound were conducted using GC-MS/MS [27].

3. Result

The result of the study demonstrated that microbial load (Bacterial, yeast and Mould) as listed in Table 1and 2and heavy metalsas depicted in Table 3 were found below the permissible concentration while aflatoxins as shown in Table 4, and pesticide residue as listed in Table 5 were not found at all.

Table 1: Microbial load in Saad Kufi (*Cyperus scariosus*)

S. No.	Microbes	Result	Permissible Limit
1.	Total Bacterial Count	3600	Not more than $1x10^5$ CFU/g
2.	Total Yeast & Mould	150	Not more than 1 $x10^3$ CFU/g

CFY/g: colony-forming units per gram

Table 2: Test for Specific Pathogens in Saad Kufi (*Cyperus scariosus*)

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

E. coli = Escherichia coli
S. aureus = Staphylococcus aureus
P. aeruginosa = Pseudomonas aeruginosa

Table 3: Heavy Metal in Saad Kufi (*Cyperus scariosus*)

S. No.	**Test parameter** (mg/kg)	Result (mg/kg)	LOQ (mg/kg)	Permissible limit (mg/kg)	Method
1.	Lead (Pb)	8.4	2.50	Not more than 10	AAS
2.	Mercury (Hg)	Not detected	0.5	Not more than 1	AAS
3.	Arsenic (As)	Not detected	1.25	Not more than 3	AAS
4.	Cadmium (Cd)	Not detected	0.25	Not more than 0.3	AAS

LOQ = Limit of Quantification
BLQ = Below the limit of Quantification
AAS = Atomic Absorption spectroscopy

Table 4: Aflatoxin in Saad Kufi (*Cyperus scariosus*)

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

LOQ = Limit of Quantification
BLQ = Below the limit of Quantification
LC-MS/MS = Lliqiuid chromatography Mass Spectrometry

Table 5: Pesticide residue in Saad Kufi (*Cyperus scariosus*)

S. No.	**Pesticides residue** (mg/kg)	Result	LOQ	Permissible Limit (mg/kg)	Method
1.	Alachlor	Not Detected	0.02	0.02	GCMSMS
2.	Aldrin & Dieldrin	Not Detected	0.04	0.05	GCMSMS
3.	Azinophos-methyl	Not Detected	0.04	1.0	GCMSMS
4.	Bromopropylate	Not Detected	0.08	3.0	GCMSMS
5.	Chlordane	Not Detected	0.04	0.05	GCMSMS
6.	Chlorfenvinphos	Not Detected	0.04	0.5	GCMSMS
7.	Chlorpyrifos	Not Detected	0.04	0.2	GCMSMS
8.	Chlorpyrifos-methyl	Not Detected	0.04	0.1	GCMSMS
9.	Cypermethrin (and isomers)	Not Detected	0.10	1.0	GCMSMS
10.	DDT (Sum of pp-DDT, pp-DDE and pp-TDE)	Not Detected	0.04	1.0	GCMSMS
11.	Deltamethrin	Not Detected	0.10	0.5	GCMSMS
12.	Diazinon	Not Detected	0.04	0.5	GCMSMS
13.	Dichlorvos	Not Detected	0.04	1.0	GCMSMS
14.	Dithiocarbamates	Not Detected	0.01	2.0	UV-VIS Spectrophotometry
15.	Endosulfan (Sum of Isomer and Endosulfan sulphate)	Not Detected	0.04	3.0	GCMSMS
16.	Endrin	Not Detected	0.04	0.05	GCMSMS
17.	Ethion	Not Detected	0.04	2.0	GCMSMS
18.	Fenitrothion	Not Detected	0.04	0.05	GCMSMS
19.	Fenvalerate	Not Detected	0.10	1.5	GCMSMS
20.	Fonofos	Not Detected	0.04	0.05	GCMSMS
21.	Heptachlor (Sum of Heptacholar & Heptachlor epoxide	Not Detected	0.04	0.05	GCMSMS
22.	Hexachlorobenzene	Not Detected	0.04	0.1	GCMSMS

23.	Hexachlorocyclohexane isomer (other than ý)	Not Detected	0.04	0.3	GCMSMS
24.	Lindane (ý-Hexachlorocylohexane)	Not Detected	0.04	0.6	GCMSMS
25.	Malathion	Not Detected	0.04	1.0	GCMSMS
26.	Methidathion	Not Detected	0.04	0.2	GCMSMS
27.	Parathion	Not Detected	0.04	0.5	GCMSMS
28.	Parathion Methyl	Not Detected	0.04	0.2	GCMSMS
29.	Permethrin	Not Detected	0.04	1.0	GCMSMS
30.	Phosalone	Not Detected	0.04	0.1	LCMSMS
31.	Piperonyl butoxide	Not Detected	0.04	3.0	LCMSMS
32.	Primiphos Methyl	Not Detected	0.04	4.0	LCMSMS
33.	Pyrethrins	Not Detected	0.10	3.0	GCMSMS
34.	Quintozen (Sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	Not Detected	0.10	1.0	LCMSMS

DDT=Dichloro diphenyl trichloroethane
DDE=Dichloro diphenyl dichloroethylene
GCMS=Gas Chromatography Mass Spectrometry
LCMS/MS = Lliqiuid chromatography Mass Spectrometry

4. Discussion

Safety studies of Unani drugs provide scientific justification for their traditional use and prove that they are safe and efficacious. Heavy metal poisoning also occurs in many cases as a result of the use of herbal medicines. A study evaluating the safety of 260 patented Asian medicines revealed that about 25% of the products contained significantly high levels of heavy metals while an addition 7% of the drugs had non recorded new compounds introduced to the drugs to generate the desired of the drugs [1]. Presence of heavy metals in a drug beyond the permissible limits cause serious side effect on brain, kidney, developing foetus, vascular and immune system [28]. Similarly aflatoxin have emerged as a major threat to human health because a number of serious side effects such as hepatotoxicity, carcinogenicity and immune suppression are associated with them. Therefore, WHO has set a permissible limit of their concentration in the plant. Although their absence in product is desirable but in case detected their concentration must be limited to the permissible limits because if the limit exceeds the drug will not be allowed to be used in the management of diseases. Standardization of Unani medicine using such practiced scientific techniques may help in building confidence for their possible use as a therapeutic medicine and their global acceptance.

5. Conclusion

All four parameters undertaken in the study are considered instrumental to determine the safety/ toxicity of drugs. Theresult of the study demonstrated that. Microbial count (Bacterial, yeast and Mould) were found below permissible limit, which is unable to produce any toxicity. Heavy metals (Arsenic, Mercury, Cadmium and Lead) were not found to be present. Aflatoxin (B1, B2, G1 and G2) cause serious side effects such as hepatotoxity, carcinogenicity etc. The absence of these toxic elements in the test drug make it safe and free from serious toxic effect. Saad Kufi (*Cyperus scariosus*) is also free from pesticide residue contamination. The findings of the present study revealed that all the safety parameters carried out on the Saad Kufi were found within the permissible limits. It indicated that the test drug can be used safely.

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