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Research Article

DETERMINATION OF HEAVY METALS, AFLATOXINS, MICROBIAL LOADS AND PESTICIDES RESIDUE IN SEHJANA (*MORINGA OLEIFERA* LAM) FRUITS/PODS

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ABSTRACT

Safety study of herbal drugs and food item is now mandatory as per WHO guidelines, to prevent the toxicity due to the material found in the soil and the environment. It includes determination of aflatoxin, heavy metal, pesticidal residue, microbial load. Therefore, the present study was aimed to evaluate safety parameter in Sehjana (*Moringa oleifera* Lam) Fruits powder, a very common drug used in Unani and Ayurvedic system of medicine for its cardiac and circulatory stimulant, and anti-oxidant, anti-inflammatory effect used in acute rheumatism, Asthma, gout, paralysis. Study reveals the presence of microbial loads, and heavy metals, lead, cadmium, mercury, and arsenic within permissible limit as per WHO guidelines, while aflatoxins, pesticides was found to be absent in the crude drug sample, indicating that the drug is free from toxicity.

KEYWORDS: Sehajana, Safety study, WHO guideline, Heavy metals, Pesticide residue.

INTRODUCTION

Moringa oleifera Lam. (family Moringaceae and genus Moringa) commonly known as Sehjana in Unani Medicine and Sahinjan in Hindi, Drumstick, and horse radish tree in English. It is medium sized tree about 10-12m height[1,2]. An extensive literature survey of Sehjana (*Moringa oleifera Lam.*) suggested that Ancient Egyptians used *Moringa oleifera* oil for its cosmetic value and skin preparation even if the species never become popular among Greeks and Romans, they were aware of its medicinal properties. Sehjana is a famous Indian drug used in a number of pathological conditions although, the entire plant has medicinal value but its fruit, leaves, seeds, have more important and interesting medicinal values. Its different parts are used after little processing as a single drug. The leaves are rich in protein, minerals, vitamins, carotene and antioxidant compounds, and other essential phytochemicals. The seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid more then 70%) and after refining a notable resistance to oxidative degradation[3].The *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, ß-sitosterol, caffeolquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant act as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic[4], antihypertensive, cholesterol lowering[5], and antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities[1,6], aphrodisiac, antihelmintic, analgesic activities[7,8], Rubefacient, Vesicant[9]. According to Unani literature it possesses many actions like Moohallil-e-Waram, Muqawwi-e-Bah, Mushtahi, Qatile Kiram-e-Amaa[10,11], so medicinally used in Waja-ul-Mafasil, Waja-ul-Qutu, Zof-e-Ishteha[12,13]. Botanically known as *Moringa oleifera Lamor Moringa Pterygosperma, Gaertn* (Moringaceae), is a fast growing softwood tree indigenous to sub-Himalayan tracts of Northern India[1]. It is one of 13 species within the same genus, and has become the most diffuse in tropical and subtropical areas at altitudes up to 2000 m[14]. It has been grown and naturalized in other countries like Pakistan, Afghanistan, Sri Lanka, Bangladesh, East and West Africa, throughout West Indies[15]. Nowadays, *Moringa oleifera* and its derivative are distributed mainly in Middle East, African and Asian countries and are still spreading to others. However, the drug has not been studied on safety parameters that are necessary to ensure its quality as suggested by WHO. Therefore, present study was undertaken to determine the presence and concentration of aflatoxins, microbial load, pesticide residue and heavy metals.

Current practices of harvesting, production, transportation and storage of herbal drugs cause additional contamination and microbial growth, proliferation of microorganisms may result from failure to control the moisture level of herbal medicines during transportation and storage[16]. Aflatoxins B_1, G_1, B_2 and G_2 are fungal secondary toxic metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius.* Aflatoxins are the strongest natural carcinogens mainly targeting the liver. The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 in the group 1 as a human carcinogen and aflatoxins G_1, B_2 and G_2 in the group B_2 as possible carcinogens[17]. Contamination of herbal materials with toxic substances such as arsenic can be attributed to many factors. And presence of heavy metals in a drug beyond the permissible limits cause serious side effect on brain, kidney, developing foetus and vascular and immune system[18].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), and soil composition and fertilizers. This contamination of the herbal material leads to contamination of the products during various stages of the manufacturing[16]. The worldwide consumption of herbal medicines has increased many folds so, it is essential to identify the risks associated with their use by large population.

MATERIAL AND METHODS

Sample preparation

The test drugs, Sehjana (*Moringa oleifera* Lam.) were collected directly from the herbal garden of department of Ilmul Advia AMU, Aligarh. And are properly identified according to the botanical, Unani and Ayurvedic literature and then confirmed in pharmacognosy section of department of Ilmul Advia. A herbarium sample of the test drugs were prepared and submitted to mawalid-e-salasa museum of the department after identification for further reference, Sehjana Voucher no, SC-0185/15. The drug was cleaned from the earthy material, washed with double distilled water and dried at 45 °C in hot air oven to powdered in electrical grinder with slow and light movement to avoid sticking of the drug material with the 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.

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 spread on 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 h, 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 Sabered glucose agar with antibiotics in a petridish of 90 mm diameter at a temperature not exceeding 45^{0}C. Alternatively the test sample was spread on 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[19].

Pesticide residue

The test for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorous compounds, and Pyrethroids compound were conducted using GC/MS-Ms[20].

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.

RESULTS

The findings in respect of the four parameters have been summarized in the tables (1-4) given below. The result of the study demonstrated that heavy metals (Arsenic, Mercury, Cadmium) were not found to be present, only Lead 7.4 mg/kg present, and microbial load count (Bacterial count 560 and Yeast and Mould 40) were found which is within permissible limit as per WHO guidelines.

Test procedures

Table 1: Heavy Metal in Sehjana (*Moringa oleifera* Lam)

Sl. No.	Test Parameter	Result (mg/Kg)	LOQ	Permissible limit (mg/Kg)
1.	Lead (Pb)	7.4	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 Quatification, BLQ = Below the limit of Quantification

Table 2: Microbial load in Sehjana (*Moringa oleifera* Lam)

Sl. No.	Microbes	Result	Permissible Limit
1.	Total Bacterial Count	560	Not more than $1x10^5$cfu/gm
2.	Total Yeast & Mould	40	Not more than 1 $x10^3$ cfu/gm

Table 3: Aflatoxin in Sehjana (*Moringa oleifera* Lam)

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

LOQ = Limit of quantification, BLQ = Below the limit of quatification

Table 4: Pesticide residue in Sehjana (*Moringa oleifera* Lam)

Sl. No.	Pesticide Residue	Result (mg/kg)	LOQ (mg/kg)	Permissible Limits (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 p,p,-DDT, p,p-DDE and p,p,-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 Endosulfansulphate)	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 (Sum of Quintozene, pentachloroaniline and methyl pentachlorophenylsulphide)	Not Detected	0.10	1.0

Table 5: Test for Specific Pathogens in Sehjana (*Moringa oleifera* Lam)

Sl. 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

Figure 1: Fruits/Pods of *Moringa oleifera* Lam.

Figure 2: Plants of *Moringa oleifera* L.

DISCUSSION

All four parameters undertaken in the study are considered instrumental to determine the safety/ toxicity of a drug. Safety studies of herbal drugs and other products used in traditional medicines have become mandatory in order to ensure their quality and risk free therapeutic application. Unani medicine is recognized as one of the safest systems of medicine because the drugs used in this system are prepared after using different procedures of purification and detoxification. Further, the temperament and pharmacokinetics of a drug are taken into account to make it commensurate with pathological conditions in which it is intended to be used. Therefore, Unani drugs in common practice scarcely produce any major side effect. However, the possibility of contamination of a drug with toxicants mainly those present in soil and atmosphere and those we use to protect the raw material from infection cannot be denied at all. Thus the crude drugs and herbal products used in Unani medicine may also contain the components of toxicants which may cause serious side effects. WHO has identified four important groups of toxic substances that should not be present in any product beyond a specified limit, as they are liable to cause very serious and life threatening effects. Therefore, it has been made mandatory to ascertain that these agents are not exceeding the permissible limits in a drug sample. Sehjana in the present study was found safe because aflatoxins, and pesticide residues were not detected, whereas the bacterial load and heavy metal were found to be many folds lower than their permissible limits. The findings indicated that the test drug is quite safe and can be used safely in the management of diseases, Since Unani single drugs undergo different procedures of purification and detoxification before being included in a preparation therefore there are chances that such toxic substances are either filtered or detoxified. Sehjana is an important and widely used drug of Unani medicine. Its pharmacological and therapeutic safety and efficacy has already been documented. However, its safety profile with respect to its quality as required by the WHO has not been recorded so far present study gives one of the earliest reports regarding its safety and being free from four important toxicants, thus it attains the required quality standards. In view of the above it can be concluded that Sehjana is a safe drug in all respect as per WHO requirement and can be used orally to manage the diseases.

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