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Original Article

Safety study of *Baobarang (Embelia ribes Burm. f.)*

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ABSTRACT

Background: *Herbal Unani drugs represent an important class of traditional medicine system. According to WHO nearly 70-80% of world population relies on traditional medicines. Herbs are used for the treatment and prevention of various ailments. With increasing interest in herbal products there is an increasing concern over the herbal drugs safety. People assume that they are natural so they are safe but there are incidences of toxicity and adverse effects. Safety study of herbal drugs and food items is now mandatory as per WHO guidelines, to prevent the toxicity due to the material found in the soil and the environment.*

Methods: *Plants are vulnerable to be contaminated with harmful ingredients. It includes determination of Microbial load, Heavy metals by AAS, Aflatoxins LC-MS/MS and Pesticide Residues using GC-MS/MS. Therefore, the present study aimed to evaluate safety parameters in Baobarang (EmbeliaribesBurm. f.) belonging to the family Myrsinaceae.*

Results: *Safety study revealed the presence of heavy metals, lead, cadmium, mercury and arsenic within permissible limit as per WHO guide line while aflatoxin, pesticides and microbial load were found to be absent in the crude drug sample.*

Conclusion: *From the safety profile obtained it can be concluded that the test drug is safe for use and free from chances of toxicity.*

Keywords: *Baobarang, Safety study, WHO Guidelines, Heavy metals, Pesticide residues*

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INTRODUCTION

EmbeliaribesBurm F a medicinal woody climber belongs to the Myrsinaceae family. It is also commonly known as false black pepper or vidanga . *E. ribes*grows in semi-evergreen and deciduous forests at an altitude of 1,500m found in central and lower Himalayas, Arunachal Pradesh, Assam, Bengal, Orissa, Andhra Pradesh and Madhya Pradesh [1]throughout India. The fruits, leaves and roots are used to cure various diseases. Baobarang fruits showed antibacterial[2] and antifertility[3] activities. It has the anti-fungal, antihelminthic and antiprotozoal properties[4]. Also in abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease and obesity[5], antifertility[6], analgesic, anti-inflammatory, antioxidant, Anthelmintic, contraceptive[7,8] Mild Appetizer, Mukhrij-e-Kirma-e-Shikam (vermifuge)[8,9] Tapeworm

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(Vermicidel)[10] Astringent, Carminative, Stimulant and tonic properties[11]Vermifuge (seeds), toothache (Roots[12] skin diseases[13,14].

Herbs normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. Current practices of harvesting, production may cause additional contamination and microbial growth of microorganism failure to control the moisture level of herbal medicines during transportation and storage[15]. Aflatoxins are naturally occurring mycotoxins that are produced principally by the strains of *Aspergillusflavus, Aspergillusparasiticus*, and also by some other species like *A. nomius*, *A. ochraceoroseus*, *A. bombycis*, *A. pseudotamari*. The four major aflatoxins B1, G1, B2, and G2 are fungal secondary toxic metabolites. Aflatoxins are the strongest natural carcinogens and their main target organ is liver. Aflatoxin B1 (AFB1) is the most potent natural carcinogen known. The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 as group I carcinogen. Ingestion of contaminated herbal plants and herbal medicines is regarded as potential source of heavy metal toxicity. Heavy metals are released into the environment by both natural and variety of anthropogenic sources. The presence of heavy metals in plant tissues is primarily dependent upon their availability and concentration in the soil. They can also be directly deposited on the plant surface from the atmosphere. Heavy metals are persistent in nature due to their long biological half-life. The major heavy metals of health concern are arsenic, cadmium, lead and mercury. Cultivation and collection of medicinal plants in the immediate vicinity of industrial area which utilizes these metals and area where these metals have been improperly disposed is highly discouraged because plants from these areas are prone to high concentration of heavy metals, hence increase the risk of contamination when taken[16,17].

METHODS

Collection and Authentication

The test drug, namely Baobarang *(EmbeliaribesBurm. f.)* was collected from Dawakhana Tibbiya college, Muslim University, Aligarh, U.P. (India). According to the botanical and Unani literature the drug was confirmed in the pharmacognosy section of Department of Ilmul Advia, A.K.T.C and in Botany Department of A.M.U, Aligarh. Then the drug was further confirmed in National Institute of Science Communication and Information Resources (NISCAIR, CSIR), New Delhi. After this an herbarium sample of the test drug was prepared and submitted to *mawalid-e-salasa* museum of the Department of Ilmul Advia, A.K.T.C, A.M.U, Aligarh for further reference.

Bobarang

Reference: Voucher No. SC-0221/17 NISCAIR
NISCAIR/RHMD/Consult/2018/3258-59-3

Finally, the drug was powdered and stored in airtight container for experimental study. The powder of Fruits of Baobarang (*EmbeliaribesBurm F*) was studied to evaluate the presence of, Heavy metals, Aflatoxin, Microbial load and Pesticide Residues at Delhi Test House, Azadpur, Delhi-110033 [QR-0302 Report No 25501709251M-78010 Sample Dated 25/09/2017 Reported on 03/10/2017]. Delhi, India.

(a) Determination of Heavy metals

The test for heavy metals is designed to determine the content of metallic impurities in the test drug. Contamination of medicinal plant materials with arsenic, lead, mercury and cadmium can be attributed to many causes including environmental pollution. Heavy metal content was determined from Delhi Test house Pvt. Ltd. by testing Protocol of ASU Guidelines (Table-1) by Atomic Absorption Spectroscopy (AAS).

(b) Aflatoxin estimation

Sample preparation

2 gm sample 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 (av), retain the supernatant, dilute 2ml of filtrate with 48 ml of phosphate buffered saline (PBS, pH7.4) to give a solvent concentration of 10% or less. The sample diluent was passed through the immune affinity column at a flow rate of 5ml/min. The column was then washed by passing 20 ml of distilled water through the column at a flow rate of approximately 5ml/min. and dried by rapidly passing through the column. 1.5 ml of distilled water was added to the sample elute. 500µl 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 2.1x150mm-5 micron, Flow=0.750ml). The aflatoxins concentration was quantified by comparing sample peak heights or areas to the total aflatoxin standard18(Table-2).

(c) Microbial load Determination

WHO has now made it mandatory to determine the microbial load in all herbal drugs used for the welfare of mankind. It was determined according to the guidelines by WHO [19](Table-3).

Method: Total Bacterial Count

The sample preparation is described below:

Pre-treatment of the test drug

Depending on the nature of the test drugs 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.

(i) For water soluble materials

10 gm of the test sample was dissolved in lactose broth (M1003-500G, Himedia labs, Mumbai, India) proven to have no bacterial activity under the condition of the test, unless otherwise specified in the test procedure for the material concerned. The volume was adjusted to 100 ml with the same medium. The pH of the suspension was adjusted to about 7.

(ii) Non-fatty materials insoluble in water

10 gm of the test sample was dissolved in the lactose broth proven to have no antimicrobial activity under the condition of the test, unless otherwise specified in the test procedure for the material concerned. A suitable surfactant-solution of Polysorbate 20R (M1307-500G, Himedia Labs, Mumbai, India) containing 1 mg/ml of Potassium tellurite (FD052, Himedia Labs, Mumbai, India) was added to aid the dilution. The volume was adjusted to 100 ml with the same medium. The pH of the suspension was adjusted to about 7.

Test procedures

Plate Count for Bacteria and Fungi

For bacteria:1 ml of the pre-treated test sample was added to about 15 ml of the liquefied casein-soybean digest agar (M290-500G,Himedia Labs, Mumbai, India) in a petridish of 90 mm diameter at a temperature not exceeding 450C. 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 30-350C 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 pre-treated test sample was added to about 15 ml of the liquefied Sabouraud glucose agar with antibiotics (MI472-500G, Himedia Labs, Mumbai, India) in a petridish of 90 mm diameter at a temperature not exceeding 450C. Alternatively the test sample was spreaded on 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.

(d) Pesticide Residues estimation

2gm test drug was taken in 5ml of ethyl acetate, extraction was made for two minutes and then centrifuged for two minutes at 10,000 rpm, the supernatant layer was taken and 1 ml of it was injected to GCMS/MS to determine the pesticide residues[20](Table-4).

RESULTS

The safety study profile of the test drug was obtained by the estimation of Microbial load, Heavy metals by AAS, Aflatoxins by LC-MS/MS and Pesticide Residues using GC-MS/MS as depicted in tables 1-4.

Table 1: Heavy Metal Analysis of Baobarang *(Embeliaribes)*

S. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Permissible Limits as API
1.	Lead as Pb (mg/kg)	9.00	2.50	Not more than 10
2.	Mercury as Hg (mg/kg)	Not Detected	0.5	Not more than 1
3.	Arsenic as As (mg/kg)	Not Detected	1.25	Not more than 3
4.	Cadmium as Cd (mg/kg)	Not Detected	0.25	Not more than 0.3

LOQ = Limit of Quantification

Table 2: Test for Aflatoxins in Baobarang

S. No.	Aflatoxins	Results	Limit of Quantification	Permissible Limit as per API
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 B_2	Not Detected	0.001	Not more than 0.1
4.	Aflatoxin G_2	Not Detected	0.001	Not more than 0.1

Table 3: Microbiological test of Baobarang

S. No.	Parameters	Test Result	Limit of Quantification	Permissible Limit as per API
1.	Total Bacterial Count (cfu/gm)	5300	--	Not more than 1×10^5cfu/g
2.	Total Yeast and Mould (cfu/gm)	220	--	Not more than 1×10^3cfu/g

Any Specific Pathogens:

1.	*E.coli /gm*	Absent	--	Absent
2.	*Salmonella/gm*	Absent	--	Absent
3.	*S.aureus/gm*	Absent	--	Absent
4.	*P.aeruginosa/gm*	Absent	--	Absent

DISCUSSION

There is a misconception about herbal drugs that people assume that as herbs are natural, they are safe but actually it is a not true so, safety study of herbal drugs and food item is now mandatory as per WHO guidelines. It includes determination of microbial load, heavy metals, aflatoxins and pesticides residues.

Table 4: Pesticidue Residue in Baobarang *(Embliaribes)*

S. No.	Pesticide Residue (mg/kg)	Results	Limit of Quantification	Permissible Limit (mg/kg)
1.	Alachor	Not Detected	0.02	0.02
2.	Aldrin and 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 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.	Dithiocarbametes (as CS2)	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	0.5
19.	Fenvalerate	Not Detected	0.10	1.5
20.	Fonofos	Not Detected	0.04	0.05
21.	Heptachlor (Sum of Heptachlor and Heptachlor epoxide)	Not Detected	0.04	0.05
22.	Hexachlorobenzene	Not Detected	0.04	0.1
23.	Hexachlorocyclohexane isomer (other than γ)	Not Detected	0.04	0.3
24.	Lindane(γ-Hexachlorocyclohexane)	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.	Piperonylbutoxide	Not Detected	0.04	3.0
32.	Primiphos Methyl	Not Detected	0.04	4.0
33.	Pyrethrins (Sum of isomer)	Not Detected	0.10	3.0
34.	Quintozen (Sum of Quitozenepentachloroanillineand methyl pentachlorophenylsulphide)	Not Detected	0.10	1.0

The contamination of herbal drugs by microorganisms not only causes deterioration, but also reduces the efficacy of herbal drugs. The toxins produced by microbes make herbal drugs unfit for human consumption because the contaminated drying may develop unwanted disease instead of disease being cured. Considerable interest therefore lies in investigation pertaining to the microbial contamination associated with drugs sample[19]. In present study herbal Unanidrugs Baobarang, was analysed for microbial load. It

includes determination of total bacterial count and total yeast and mold count. As per WHO norms, the total bacterial count by serial dilution method was found to be within perimissible limit in the drug sample. The specific pathogenic bacteria (*Enterobacteriacea, E. Coli, Salmonella sp., Pseudomonas aeruginosa* and *Shigella*) were absent in the sample of test drugs. The result of present study is summarized in Table-1

The medicinal plants contain varying amounts of various heavy metals. They may be due to contamination or some plants absorb from atmosphere. These could be both essential as well as non-essential. The excess of trace metal can cause serious toxic effects on health. It is important to have good quality control practice of herbal product and standardized extract screening in order to protect consumer from toxicity [20] Heavy metal contamination of test drugs was determined by Atomic Absorption Spectrometry (AAS) method and was found to contain Lead with in permissible limits Mercury, Arsenic and Cadium were absent (Table-2).

Aflatoxins are a group of mycotoxins that are produced mainly by member of the genus *Aspergillus.* Production of these toxic secondary metabolites is closely related to fungal development [21]. Contamination of food, feed and agriculture commodities by aflatoxins possess enormous economic and serious health concern because these chemicals are highly carcinogenic and can directly influence the structure of DNA[22], these are the strongest natural carcinogens and their main target organ is Liver. The International Agency for Research on Cancer (IARC) has classified aflatoxins B_1 as carcinogenic and aflatoxoinG1,B2 and G2 are possible carcinogens to humans . Aflatoxin B1, B2, G1 and G2 are highly contaminants in any material of plants origin[20], and thus screening of test drugs for aflatoxin was conducted by Liquid Chromatography -Mass spectrometry (LCMS/MS) showed that aflatoxin was absent in the test drug sample (Table-3).

Herbal drugs are liable to contain pesticide residue which accumulate from agriculture practice, such as spraying, treatment of soil during cultivation, and administration of fumigants during storage. However, it may be desirable to test herbal drugs for broad group in general, rather than for individual pesticide. Sample of herbal material were extracted by a standard procedure, impurities were removed by partition or absorption, and individual pesticides were measured by GC-MS[20]. The pesticides residues for test drugs were showed within normal limits as summarized in (Table-4).

CONCLUSION

The safety profile determination of every finished product has been made mandatory by WHO. The safety studies for the determination of microbial load. Heavy metal contamination by lead, cadmium, mercury and arsenic, aflatoxins contamination and pesticide residues were done, and it was found that the test drug was harmless, and the values so obtained were within the allowed limit of each. So, it can be concluded that the test drug is safe for use and free from chances of toxicity.

VI. FUTURE SCOPE

Safety profile helps in establishing the safety and toxicity of herbal drugs from contaminants like heavy metals, microbes, aflatoxins and pesticide residues.

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