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Safety study of a single Unani drug: Gul-e-Ghafis (*Gentiana kurroo royle*)

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Abstract

Plants have been used to treat and prevent ailments since prehistoric times, one of the most important sources of medication is plants, and about 80% of the world's population is using medicinal plants as a source of fibre and as a source of medicine. Unani drugs are of keen interest for research in many aspects including prophylactic role, efficacy in diverse diseases and safety. Recent study is was aimed to evaluate the safety parameters of Gul-e-Ghafis (*Gentiana kurroo royle*) a very common unani drug used in inflammation and bitter tonic. WHO has mandatory to do safety study of herbal drug and food items before performing any experiments as per their guidelines. It comprises determination of heavy metals, aflatoxin, pesticidal residue, microbial load. Study reveals the presence of heavy metal, lead, mercury cadmium and arsenic within normal limit as per WHO guidelines while aflatoxin, pesticide and microbial load was found to be absent in crude drug sample. It can be said that Ghafis is free from toxicity and used safely for human use.

Key words: ailments, Gul-e-Ghafis, bitter tonic, aflatoxin, arsenic.

Introduction :

Plants have been used to treat and prevent ailments since prehistoric times, The Unani system of medicine is an age old but time tested system of health care. Along with the significant
increase of worldwide consumption, the safety of herbal medicine has been highlighted. It is assumed by the people that herbal medicines can be taken in a long term and has no toxin or adverse effect so forth because these medicines are originated from nature and belongs to green therapy, but it should be clearly recognized that as compared with synthetic drug herbal medicinal products are widely considered to be of lower risk but they are not completely free from the possibility of toxicity or adverse effects. Therefore, to

ensure the safety of herbal medicinal products it is mandatory to do safety study as per WHO guidelines. [1] It includes determination of heavy metals, aflatoxins, microbial load and pesticide residues. The efficacy of herbal medicine is reduced if it is contaminated with microorganisms, as well as it causes deterioration of such medicine which make herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of being cured, due to toxins produced by microbes. Considerable interest therefore lies in investigation pertaining to the microbial contamination associated with the drugs sample. [2] It includes determination of total yeast, bacterial count and mould count.

There are varying amounts of heavy metals present in medicinal plants due to contamination or some plants absorb them from atmosphere. Presence of these could be essential in limited amounts but in excess, these may cause serious toxic effects on health. It is important to have good quality control practice of herbal product and screening of standardized extracts in order to protect consumer from toxicity. [3, 4] Heavy metal contamination of test drugs was determined by Atomic Absorption Spectrometry (AAS) method and was found to contain Lead, mercury, Arsenic, Cadmium within permissible limits. Aflatoxins are a group of mycotoxins that are produced mainly by members of the genus *Aspergillus.* Production of these toxic secondary metabolites are closely related to fungal development. [5] Today's major health concern are related to these chemicals due to their carcinogenic properties that can directly influence the structure of DNA. [6] The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 as carcinogen and aflatoxin G1, B2 and G2 are possible carcinogens to humans. [7] Aflatoxin B1, B2, G1 and G2 are highly toxic contaminants in any material of plant origin. [8] and thus screening of test drugs for aflatoxin was conducted by Liquid Chromtography - Mass Spectrometry (LCMS/MS).

From cultivation to storage, malpractice of agriculture like soil treatment, administration of fumigations, pesticide residue accumulate in herbal drugs. So herbal drugs were tested for broad groups in general, rather than individual pesticide. Sample of herbal material were extracted by a standard procedure, impurities were removed by partition or adsorption and individual pesticides were measured by GC-MSMS. [3]

Present study is an attempt to assess these safety profile of a well known herbal drug used in Unani system of medicine Gul-e-Ghafis (*Gentiana kurroo Royle*).

Gul-e-Ghafis (flowers of *Gentiana kurroo Royle*) is one of the important herbal drug used from ancient period of time in Unani system of medicine belongs to the family Gentianaceae.[9] It is small perennial herb, up to 30 cm height, with tufted decumbent stems It is a critically endangered medicinal plant species, endemic to the northwestern Himalayas. [10] Dioscorides reported that it has inverted fruit, on ripening they acquire tendency for adherence to the clothes. *Gentiana kurroo Royle* was known by the name of Shajrat-el-baragith and Shaukat-el-muntineh by the western Arabs later on, the name was changed as Ghafith or khafil. In the eastern region this plant is still found by the eastern Arabs under the name of Ghafis or Ghafith. Barhalaan is another name of Ghafis given by eastern Arabs, where as in Kitabul-Hashaish, Dioscorides has said, Barhalaan as Quateera in third chapter of his book and in Arabic it is known as Tabaaq.[11] The common name of Gentiana has been derived from "Gentius" a king of Illyria (Europe), who discovered the medicinal value of the Gentian root. In fact, the specific name of *G. kurroo Royle* was

derived from the local name of the root of the plant, "Karu" meaning bitter. *Gentiana kurroo Royle* commonly known as "Indian Gentian" or Himalayan Gentian in English, "Karu" in Hindi, Nilkanth in Kashmir Himalaya. [12] Ghani in khazain-ul advia reported that Ghafis grow in hilly areas of Shiraz is better than in Rome but Romy variety is not so bad. Its potency can persist upto 3 years.It is cultivated by seed propagation, cutting and root division.[13] leaves are narrowly oblong-lanceolate or elliptic-lanceolate radical, greenish black 3-13.5x0.3-1.5 cm. The roots and rhizomes are brownish, sub cylindrical having wrinkled surface. It is brittle and breaks with a short fracture, at first the taste is sweet and intensely bitter afterwards The fruits are achene type.[14, 15]

Dull, brown powder having bitter taste, consists of abundant smooth pollen grains, fragments of endothelial cells of anther, brownish fragments of petal showing epidermal cells with wavy wall, spiral and scalariform like thicknings of trachieds, druses and prismatic crystals of calcium oxalate are also present [16] . In Unani literature actions described as Mufatteh-e- sudad (Deobstruent), Mudirr-e-Haiz (Emmenagogue), Mudirr-e-Baul (Diuretic), Qabiz (Astringent), Muqawwi-e-Meda (Stomachic), Muwallid-e-Sheer (Galactagogue), Da'afa-e-Zahar (antidote), Jali (Detergent), Musaffi Dam (Blood purifier), Muarriq (Sudorific)

Mushil-Akhlat-e-Hadda (purgative to corrosive Humors), Da'afa-e-Humma (Anti-Pyretic)

Muqawwi (Tonic), Qatil-e –Deedan (Taenniacide), Da'afa-e-scurvy (Anti-scorbutic)

Muqawwi-e-jigar (Liver Tonic), Muhallil-e- Auram (anti-Inflammatory), Mudirr-e –Safra (Cholagogue), Mujaffif-Wa-Mudammil-e-Qurooh (Cicatrizant). It is used in Awram-e-jigar wa Tehal (Inflammation of Liver and Spleen), Humma Ha'ad (acute fever), Humma Kuhna (Chronic fever), Humma ruba (Quartan fever), Qurooh khabisa (Non healing Ulcer), Yarqan (Jaundice), Istisqa (Ascites), Saufa (Alopecia), Qurooh (Wounds), Sudade-e-Jigar wa Tihal (Hepatic and splenic obstruction), Ishal (Simple diarrhoea), Usre Baul (Dysuria), Jarb (Scabies), Daussadaf (Psoriasis), Seborrheic dermatitis. [9, 11, 13, 14, 15]

Anti Alzheimer activity of methanolic extract was revealed by Sajjad *et al*; 2019 in mice. The decoction of the flower tops of *Gentiana kurroo Royle* showed antiinflammatory activity against the inflammation induced by carrageenan in acute phase explained by latif et al. Many scientists have suggested that Methanolic extract *Gentiana kurroo Royle* is well known for its immunomodulatory activity, when it was administered to the mice. *Gentiana kurroo Royle* possess alkaloids, amino acids, gentimic, cardiac glycosides, flavonoids, glycosides, Phenols, proteins, sterols/terpenes, reducing sugars and non reducing sugars and tannins. flavonoids and carbohydrates, many Iridoid glycosides namely 6-cinnamo-yl-catalpol, 6-O-cinnam-yl catalptol, 6-O-vanillyl, 6-O-feruloyl catalpol, catalpol (335) and aucubin. The root of *Gentiana kurroo Royle* also possess gentianic acids, gentian bitter, and uncrystallizable sugar [17, 18, 19, 20]

Material and method:

The test drug namely Gul-e Ghafis (*Gentiana kurroo Royle*) flowers were collected from Dawakhana Ajmal khan Tibbiya college. All the drugs were properly identified according to the botanical and Unani literature and then confirmed in pharmacognosy section of Department of Ilmul Advia, A.K.T.C and in

Botany department of A.M.U, Aligarh. The herbarium samples 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 after identification for further reference with Voucher no. SC-0323/22.

Before carrying out safety study the crude test drug Gul-e- Ghafis procured were dried in shade and stored in an air tight container. However before subjecting to pulverization they were again dried in hot air oven at a temperature not exceeding 40°C. They were powdered with the help of an electric grinder at pharmacy lab of department of Ilmul Advia. To get a relatively fine powder of uniform particle size it was passed through a sieve no.80.

Safety Study

The test drug was studied to evaluate the presence of heavy metals, microbial load, pesticide residues and aflatoxins. The determination of these parameters was carried out at "Delhi Test House, Azadpur, New Delhi 110033 India, [QR 0302 Report no, 23102111271M68014 sample dated 21/11/2021, Reported dated 9/12/2021] using the following parameters.

(I) Microbial load Determination

W.H.O has now made it mandatory to determine microbial load in all herbal drugs used for the welfare of mankind. It was determined according to the guidelines by W.H.O

Method: Total Bacterial Count

The sample preparation 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

(a) 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 (M290-500G, Himedia Labs, Mumbai, India) in a petridish of 90 mm diameter at a temperature

not exceeding 45°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°C for 48-72 hours, unless a more reliable count was obtained in a short period of time. The number of colonies so formed were 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 45°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°C for 5 days, unless a more reliable count was obtained in a short period of time. The number of colonies so formed were counted and the results were calculated using the plates with not more than 100 colonies.

(II) Determination of Heavy metals

The test for heavy metals is designed to determine the content of metallic impurities in the test drugs. 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 by AAS, from Delhi Test house Pvt. Ltd. by testing Protocol of ASU Guidelines

(III) 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, pH 7.4) 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 to the LC-MSMS (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 standard. [21]

(IV) Pesticidal Residue estimation

2 gm test drug was taken in 5 ml 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 GC-MSMS to determine the pesticidal residue. [22]

Result:

The result of the study demonstrated that Heavy metals contamination i.e. Pb, As, Hg and Cd in the test drug was within permissible limits as shown in Table 1. Total microbiological count (Bacterial, Yeast and

Mould) were found to be within negligible limit in the test drug sample as listed in Table 2. The specific pathogenic bacteria *E.Coli, Salmonella sp*., and *Pseudomonas aeruginosa* were absent in drug sample of herbal test drug as depicted in Table 3. Screening of test drug for aflatoxins determination showed that aflatoxins were absent in the test drug sample as shown in Table 4. The given test drug showed pesticides residue as shown in Table 5 were not found at all.

Table: 1 Heavy Metal Analysis of Gul-e-Ghafis (*Gentiana kurroo Royle*)

S. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Permissible Limits
1.	Lead as Pb	Not detected	2.5 ppm	Not more than 10 ppm
2.	Mercury as Hg	Not detected	0.5 ppm	Not more than 1 ppm
3.	Arsenic as As	Not detected	1.25 ppm	Not more than 3 ppm
4.	Cadmium as Cd	Not detected	0.25ppm	Not more than 0.3 ppm

LOQ = Limit of Quantification

Table: 2 Microbiological test of Gul-e-Ghafis (*Gentiana kurroo Royle*)

S. No.	Parameters	Test Result	Permissible Limit
1.	Total Bacterial Count (cfu/gm)	3900	Not more than 1×10^5 cfu/g
2.	Total Yeast and Mould (cfu/gm)	280	Not more than 1×10^3 cfu/g

cfu/gm = Colony- forming unit per gram

Table:3 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

E.coli = Escherichia coli

S. aureus = Staphylococcus aureous

P.aeruginosa = Pseudomonas aeruginosa

Table: 4 Test for Aflatoxins in Gul-e-Ghafis (*Gentiana kurroo Royle*)

S. No.	Aflatoxins	Results	Permissible Limit
1.	Aflatoxin B_1	Not detected	Not more than 0.5 ppm
2.	Aflatoxin G_1	Not detected	Not more than 0.5 ppm
3.	Aflatoxin B_2	Not detected	Not more than 0.1 ppm
4.	Aflatoxin G_2	Not detected	Not more than 0.1 ppm

Table: 5 Pesticidal Residue in Gul-e-Ghafis (*Gentiana kurroo Royle*)

S. No.	Pesticide Residue	Results	Limit of Quantification	Permissible Limit (mg/kg)
1.	**Chlorpyrifos**	Not detected	0.04	0.2
2.	**Chlorpyrifos-methyl**	Not detected	0.04	0.1
3.	**DDT (Sum of p.p-DDT, p.p.-DDE & p.p.-TDE)**	Not detected	0.04	1.0
4.	**Endosulfan (Sum of Isomer & Endosulfan Sulphate)**	Not detected	0.04	3.0
5.	**Malathion**	Not detected	0.04	1.0
6.	**Parathion**	Not detected	0.04	0.5
7.	**Parathion Methyl**	Not detected	0.04	0.2
8.	**Alachlor**	Not detected	0.02	0.02

9.	**Aldrin and Dieldrin (sum of)**	Not detected	0.02	0.05
10.	**Azinophos-methyl**	Not detected	0.04	1.0
11.	**Bromopropylate**	Not detected	0.08	3.0
12.	**Chlordane(sum of cis,trans and oxychlordane)**	Not detected	0.04	0.05
13.	**Chlorphenvinphos**	Not detected	0.04	0.5
14.	**Chlorpyrifos**	Not detected	0.04	0.2

Discussion:

As drugs are related with the life of a person, so safety plays a major role in case of herbal drugs. WHO has made the safety profile of every finished product mandatory, whether it is single or compound drug? Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi and viruses, which depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Therefore, safety study for determination of microbial load, heavy metal contamination by lead, mercury, cadmium and arsenic, aflatoxin contamination and pesticidal residue was done.

Conclusion:

It was found that the test drug is not harmful for use of medicinal purpose and the value obtained were within the permissible limit, so it is concluded from this study that the test drug is safe, free from toxicity and hazardous effects.

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