Safety Study of *'Qurs-e-Ziyabetus'*–A Unani Pharmacopoeial Compound Formulation

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Abstract

Plant drugs used in traditional medicines are liable to be contaminated with toxic substances. Plants are prone to be contaminated with them during the agricultural practices and thereafter and could lead to poisoning, besides side effects like depression, memory, loss of sensation and chronic renal failure etc. Contamination of herbal products is a public health issue of global significance and the use of these products may be a risk for toxicity of heavy metals and other toxicants. Therefore, safety studies of the herbal drugs are now mandatory as per WHO guidelines. It includes Aflatoxin determination, Heavy metal analysis, Pesticidal residue evaluation, and Microbial load determination. Although drugs under the Indian System of Medicine (ISM) are required to be manufactured in hygienic environment following the GMP norms after quality assurance of the raw materials still the products are tested for the toxicant mentioned above in order to ensure their safety and efficacy. Therefore, in present study, the powder of Qurs-e-Ziyabetus was studied on safety parameters.

The test drug (QZ) showed that all the safety parameters were found within the permissible limits as per WHO guidelines; hence we can say that our test drug Qurs-e-Ziyabetus (QZ) is quite safe and does not contain the toxic materials.

Key words: Safety study, *Qurs-e-Ziyabetus*, Unani drug

Introduction

In Unani system of medicine a number of single drugs and compound formulations are used in the management of Diabetes. *Qurs-e-Ziyabetus* (QZ) is an important compound preparation mentioned in various Qarabadeen (Pharmacopoeias and Formularies) with little variation of ingredients; though all the preparations are used for same therapeutic effect. The formulation under study however has been taken from Qarabadeen Aazam wa Akmal (Akmal, ynm) in view of its wide acceptability among the physicians who prescribe it in their routine practice to manage the diabetes and the conditions associated with it.

QZ includes a number of single herbal drugs (Table 1) as its ingredients that have been attributed to possess different pharmacological effect that may ameliorate the diabetic condition as such or induce such a response that may negotiate the complications arise out of diabetes. Since the management of diabetes requires inclusion of drugs in a combination that may address the need of lowering glucose level and other symptomatic and systemic relief therefore this combination appears to be comprehensive as it includes the drugs that have been described to reduce the glucose level and polyuria (Hafeez, 2005). As per Unani description the physiopathology arising mainly from kidney but also evolving spleen and

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liver etc along with certain metabolic disturbances, give rise to diabetes mellitus. Further, the disease over a period of time affects almost all organs and systems of the body vitiating the human health. Therefore a compound drug having different pharmacological effects to deal with diabetes and its complications appears to be more suitable as compared to single drug or a molecule. Some of the drugs described to strengthen the kidney and visceral organs improve the metabolism, reduce the thirst and have general tonic effect have been included in the test drug for wide therapeutic requirements. Ability of compound drugs to deal with complex problems notwithstanding being appreciable is sometimes undermined because of their poor quality standards, as any ingredient of inferior quality may spoil the measures of the entire product. The chances of contamination with different toxic substances are also high in compound preparations. Therefore, WHO has set a standard for herbal drugs and their products in respect of their quality and safety. Various constants of safety have been made mandatory to put within the permissible limits including aflatoxin, heavy metals, microbial loads and pesticide residues. Every product is therefore tested to determine the four constant of safety parameters.

Table 1: Ingredients of 'Qurs-e-Ziyabetus'

S. No	Ingredients	Botanical name	Quantity
1.	Tabasheer	*Bambusa arundinacea*	35 gm
2.	Rubbussus	*Extract of liqueorce*	35 gm
3.	Tukhm-e-kahu	*Lactuca sativa*	70 gm
4.	Tukhm-e-khurfa	*Portulaca oleracea*	52.5 gm
5.	Gil-e-armani	*Bole armeniae rubra*	17.5 gm
6.	Gul-e-surkh	*Rosa damascena*	17.5 gm
7.	Kishneez khushk	*Coriadrum sativum*	17.5 gm
8.	Samagh-e-arbi	*Acacia arabica*	7 gm
9.	Sandal safed	*Santalum album*	7 gm
10.	Sandal surkh	*Pterocarpus santalinum*	7 gm
11.	Gulnar	*Punica granatum*	2 gm
12.	Kaphur	*Cinnamomum camphora*	1.75 gm

The test drug despite being used commonly in clinical practice and being standardized on physicochemical parameters 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.

Medicinal plants may get contaminated easily by absorbing heavy metals from soil, water and air. Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point sources including different industrial activities. Additional sources of these elements for plants are rainfall, atmospheric dusts and plant protection agents (Nema, 2016). Herbal 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 microorganisms may result from failure to control the moisture level of herbal medicines during transportation and storage (Anonymous, 2007). 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 2B as possible carcinogens (Meritxellventura *et al*., 2004). Contamination of herbal materials with toxic substances such as arsenic can be attributed to many factors. Toxic elements from waste water may contaminate agricultural soil, water supply and environment. These toxic metals confined in plants finally enter the human body and may disturbs the normal functioning of central nervous system, liver, lungs, heart, kidney and brain leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of malignancies (Nema, 2016; Anonymous, 2007).

The worldwide consumption of herbal medicines is enormous. So, it is essential to identify the risks associated with their use as safety of herbal medicines is an important public health issue (Anonymous, 2004). In view of the above, the present study was undertaken to prepare the safety profile of QZ.

Material and Method

Sample preparation

The crude drugs were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh. After getting confirmation of purity and identity from Pharmacognosy section of Department of Ilmul Advia, all the ingredients were powdered in an electric grinder separately and mixed together in equal proportion as mentioned in the Pharmacopeia. The mixed drug was then passed through the sieve no. 80 to get equally fine powder. It was stored in an air tight container for further experiments.

Powdered test drug was studied to evaluate the presence of Microbial load, Pesticides residue, Aflatoxins and Heavy metals.

Microbiological determination Test

Total viable aerobic count (TVC)

The total viable aerobic count (TVC) of the test drug was determined using Plate Count Method.

Pretreatment of the test drug

Compound formulation was dissolved and antimicrobial property present in the sample, if any was eliminated by dilution or neutralization. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G), Himedia Labs, Mumbai, India, was used for diluting the test sample.

Plate count for bacteria

1 ml of the pretreated test sample was added to about 15 ml of the liquefied casein-soybean digest agar in a petri dish of 90 mm diameter at a temperature not exceeding 45 ºC. 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 ºC for 48-72 hours, 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 (Lohar, 2007).

Test for pesticide residue

The test for the assessment of specific pesticide residues like organochlorine compounds, organ phosphorous compounds and pyrethroids compounds was done using GCMS-MS (Ramkrishanan *et al*., 2015).

Test for Aflatoxins

LCMS-MS was used to determine the different Aflatoxins including B_1, G_1, B_2 and G_2 (Maritxellventura *et al*., 2004).

Test for heavy metals

Heavy metals like Arsenic, Mercury, Cadmium and Lead, beyond the permissible limit affect the health and produce adverse effect on brain, kidney, developing foetus, normal growth, vascular and immune system (Moses and Moebe, 2012). This test was conducted using AAS technique.

Observations and Results

The results of the four tests have been presented in the following tables (1-4):

Table 1: Heavy Metals in Qurs-e-Ziyabetus

S. No	Test Parameter	Result (mg/ kg)	LOQ (mg/kg)	Permissible limit (mg/kg)
1	Lead (Pb)	9.4	2.50	NMT 10
2	Mercury (Hg)	Not detected	0.5	NMT 1
3	Arsenic (As)	Not detected	1.25	NMT 3
4	Cadmium (Cd)	Not detected	0.25	NMT 0.3

Table 2A: Microbial load in Qurs-e-Ziyabetus

S.No.	Test for Microbiology	Result (cfu/gm)	Permissible Limit (cfu/gm)
1	Total Bacterial Count	600	NMT 10^5
2	Total Yeast and Mould	<10	NMT 10^3

Table 2B: Microbial load in Qurs-e-Ziyabetus

S.No	Specific Pathogen	Result (/gm)	Permissible limits as per API
1.	*E. Coli*	Absent	Absent
2.	*Salmonella*	Absent	Absent
3.	*S. aureus*	Absent	Absent
4.	*P. aeruginosa*	Absent	Absent

Table 3: Aflatoxin in Qurs-e-Ziyabetus

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

Table 4: Pesticidal residue in Qurs-e-Ziyabetus

S.No	Pesticide Residue	Result	LOQ (mg/kg)	Permissible limit (mg/kg)
1	Alachor	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	Cholordane	Not detected	0.04	0.05
6	Chlorfenvinphos	Not detected	0.04	0.2
7	Cypermethrin (and isomers)	Not detected	0.10	1.0

8	Chlorpyrifos	Not detected	0.04	0.2
9	Chlorpyrifos-methyl	Not detected	0.04	0.1
10	DDT (Sum of p.p-DDT, p.p-DDE and p.p-TDE)	Not detected	0.04	1.0
11	Lindane	Not detected	0.04	0.6
12	Deltamethrin	Not detected	0.10	0.5
13	Diazinon	Not detected	0.04	0.5
14	Dichlorvos	Not detected	0.04	1.0
15	Dithiocarbamates(as CS2)	Not detected	0.01	2.0
16	Endosulfan (Sum of Isomer and EndosulfanSulphate)	Not detected	0.04	3.0
17	Endrin	Not detected	0.04	0.05
18	Ethion	Not detected	0.04	2.0
19	Fenitrothion	Not detected	0.04	0.5
20	Fenvalerate	Not detected	0.10	1.5
21	Fonofos	Not detected	0.04	0.05
22	Heptachlor (Sum of Heptachlor & Heptachlor epoxide)	Not detected	0.04	0.05
23	Hexachlorobenzene	Not detected	0.04	0.1
24	Hexachlorocyclohexane isomers	Not detected	0.04	0.3
25	Malathion	Not detected	0.04	1.0
26	Parathion	Not detected	0.04	0.5
27	Methidathion	Not detected	0.04	0.2
28	Parathion Methyl	Not detected	0.04	0.2
29	Piperonyl butoxide	Not detected	0.04	3.0
30	Primiphos Methyl	Not detected	0.04	4.0
31	Permethrin	Not detected	0.04	1.0
32	Pyrithrins (Sum of isomers)	Not detected	0.10	3.0
33	Phosalone	Not detected	0.04	0.1
34	Quintozen (Sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphate)	Not detected	0.10	1.0

Discussion

All the four parameters undertaken in the study to determine the safety of the test drug serve as important tools of quality control and standardization. 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. About 80% of populations worldwide rely on herbal medicines for their primary healthcare requirements. Adverse effects associated with herbal medicines may result from contamination of products with toxic metals; adulteration, misidentification, substitution of herbal ingredients or improperly processed or prepared products. 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. Therefore commonly it is believed that Unani drugs do not produce any major side effects. However, the possibility of contamination of herbal medicine with toxicants by absorbing heavy metals from soil, water and air cannot be denied. Usually soil is subjected to contamination through atmospheric deposition of heavy metals from different industrial activities. Additional sources of these elements for plants are rainfall, atmospheric dusts and plant protecting agents. Toxic elements from waste water may contaminate agricultural soil, water and environment. Finally these toxicants enter the human body and may disturb its normal functioning and cause a number of serious side effects such as hepatotoxicity, carcinogenicity and immune-suppression etc. Therefore, it has been made mandatory to ascertain that these agents are not exceeding the permissible limits in test drug. QZ in the present study was found safe because aflatoxins, pesticide residues were not detected at all in the test sample, whereas the bacterial load was found to be many folds lower than their permissible limits. Similarly the heavy metals were also found within the permissible limits. The findings indicated that the test drug is quite safe and can be used effectively in the management of diseases. The study provides one of the earliest reports about the safety of QZ. The individual ingredients and to some extent the composition has been studied earlier for various pharmacological and standardization related profiling. By providing the data regarding its safety profile the drug can now said to be effective and safe and can be used therapeutically without a fear of serious toxicity.

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