Safety Study of 'Majoon-e-Gul' – A Unani Pharmacopoieal Compound Formulation

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Abstract

Safety study of herbal drugs and food items is now mandatory as per WHO guidelines. It includes determination of aflatoxin, heavy metals, pesticidal residue and microbial load. Plants are more vulnerable to be contaminated with these components during the agricultural practices and thereafter. Therefore, studies to determine the presence / absence of these components to decide the toxicity / safety of drug is necessary. In the present study, the ingredients of Majoon-e-Gul (MG) except its base i.e. sugar and vinegar were studied on safety parameters.

All the safety parameters were found below the permissible limit as per WHO guidelines, indicating that Majoon-e-Gul is free from toxicity.

Keywords: Majoon-e-Gul, Safety study, Heavy metals, Aflatoxins, Pesticide residue, Microbial load.

Introduction

Unani System of Medicine possess a number of formulations and single drugs which are used in the management of liver disorders for a long time. Majoon-e-Gul (MG) is one of the important compound preparations mentioned in National Formulary of Unani Medicine (Anonymous, 2007) and other Qarabadeen which is used in liver ailments like Hepatitis (*Warm-e-jigar)*, Jaundice (*Yarqaan)* etc. (Anonymous, 2007). It has been shown to produce hepatoprotective and anti-hepatitis effect against Carbontetrachloride (CCl_4) induced hepatic damage. It contains 8 ingredients (Table 1) including sugar and vinegar. However, the study was conducted on the ingredients other than sugar and vinegar in powder form.

Table 1: Ingredients of Unani Pharmacopoeial Compound Formulation 'Majoon-e-Gul'

S.No.	Scientific name	Unani name
1.	*Rosa damascena* Mill	Gul-e-Surkh
2.	*Crocus sativus* Linn	Zafran
3.	*Rheum emodi* Wall ex Meissn	Rewand Chini
4.	*Irsa ensata* Thunb.	Irsa
5.	*Cinnamomum cassia* Blume	Saleekha
6.	*Coccus lacca*	Laak
7.	Sugar	
8.	Vinegar	

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These ingredients are mixed in a base prepared with sugar to make a semi solid preparation (Majoon). However, due to increasing incidence of diabetes and dietary restrictions warranted in a number of metabolic and other diseases the use of the drugs prepared in sugar bases is not desirable. Therefore, MG's hepatoprotective, curative and hepatocytes regenerative effects are attributed to its antioxidant activity (Parveen *et al*., 2009).

Cause of contamination of foods & water and herbal materials

All the ingredients of MG have been described to have hepatoprotective, resolvent, anti-inflammtory, astringent and diuretic activity and few have also shown anti-cancerous activity (Nadkarni, 2010). 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 levels 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 and their main target organ is 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 to humans (Meritxell Ventura, 2004). 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 runoff water which finds its way into rivers, lakes and the 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 (Anonymous, 2007). 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 (Anonymous, 2004). In view of the above, the safety study of test drug Mazoon-e-Gul, has been conducted so as to prepare its safety profile.

Material and Methods

The study was conducted in the Department of Ilmul Advia, A.K. Tibbiya College, Aligarh, during 2015.

Sample preparation

The ingredients of MG were procured from Dawakhana Tibbiya College, Aligarh. After the confirmation of purity and identity of each of the ingredients from

pharmacognosy section, all these, except Zafran, were powdered in an electric grinder, separately. Zafran was powdered in dry mortar (kharal) with slow and light movement of pestle to avoid sticking of the drug material with the mortar and there after all the ingredients were powdered and mixed together in a proportion as mentioned in the Unani Pharmacopeia. After mixing of all the ingredients 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.

Powdered 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, total viable aerobic count (TVC) of the compound formulation was determined, as specified in the test procedure, using plate count.

Pre-treatment of the compound formulation

Depending on the nature of the compound 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.

Test procedures

1. 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 hrs, 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 pretreated 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 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 (Lohar, 2007).

2. Pesticidal residue

The test for the assessment of specific pesticide residues like Organochlorine compounds, Organophosphorous compounds and Pyrethroids compounds were conducted using GCMS-Ms (Ramkrishanan, 2015).

3. Aflatoxins

The test for determination of the aflatoxins was performed using LCMS-MS.

4. Heavy metals

Heavy metals including Arsenic, Mercury, Cadmium and Lead were determined in the test sample using Atomic Absorption Spectroscopy.

Table 2: Heavy Metals in MG

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

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

Table 3: Microbial load in MG

S.No.	Microbes	Result	Permissible Limit
1.	Total Bacterial Count	10000 cfu/g	Not more than 10^5 cfu/g
2.	Total Fungal Count	<10 cfu/g	Not more than 10^3cfu/g

Table 4: Aflatoxin in MG

S.No.	Aflatoxin	Result	LOQ (mg/kg)	Permissible Limit (mg/kg)
1	Aflatoxin B_1	BLQ	0.0005	Not more than 0.50
2	Aflatoxin G_1	BLQ	0.0005	Not more than 0.50
3	Aflatoxin G_2	BLQ	0.0005	Not more than 0.10
4	Aflatoxin B_2	BLQ	0.0005	Not more than 0.10

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

Table 5: Pesticidal residue in MG

S.No.	Pesticide Residue	Result (mg/kg)	LOQ (mg/kg)	Permissible Limits (mg/kg)
1	Alachor (mg/kg)	Not detected	0.02	0.02
2	Aldrin & dieldrin (mg/kg)	Not detected	0.04	0.05
3	Aziphos-methyl (mg/kg)	Not detected	0.04	1.0
4	Bromopropylate (mg/kg)	Not detected	0.08	3.0
5	Cholordane (mg/kg)	Not detected	0.04	0.05
6	Chlorpyriphos (mg/kg)	Not detected	0.01	0.2
7	Chlorpyriphos-methyl (mg/kg)	Not detected	0.01	0.1
8	Cypermethrin (mg/kg)	Not detected	0.10	1.0
9	DDT (Sum of p.p-DDT, p.p-DDE and p.p-TDE)	Not detected	0.01	1.0
10	Deltamethrin (mg/kg)	Not detected	0.10	0.5
11	Diazinon (mg/kg)	Not detected	0.04	0.5
12	Dichlorvos (mg/kg)	Not detected	0.04	1.0
13	Dithiocarbamates (mg/kg)	Not detected	0.01	2.0
14	Endosulfan (Sum of Isomer and Endosulfan Sulphate)	Not detected	0.04	3.0
15	Endrin (mg/kg)	Not detected	0.04	0.05
16	Ethion (mg/kg)	Not detected	0.04	2.0
17	Fenitrothion (mg/kg)	Not detected	0.04	0.5
18	Fenvalerate (mg/kg)	Not detected	0.10	1.5
19	Fonofos (mg/kg)	Not detected	0.04	0.05
20	Heptachlor (mg/kg)	Not detected	0.04	0.05
21	Hexachlorobenzene (mg/kg)	Not detected	0.04	0.1
22	Malathion (mg/kg)	Not detected	0.04	1.0
23	Parathion (mg/kg)	Not detected	0.04	0.5
24	Parathion Methyl (mg/kg)	Not detected	0.04	0.2
25	Permethrin (mg/kg)	Not detected	0.04	0.5
26	Phosalone (mg/kg)	Not detected	0.04	0.1

Discussion and Conclusion

All the four parameters undertaken in the study are considered instrumental to determine the safety /toxicity of a drug. They serve as important tools of quality control and standardization and have been proposed as mandatory requirement for plant drugs by WHO. The findings of the study demonstrated that four heavy metals namely arsenic, mercury, cadmium and lead and four different aflotoxins were not found to be present. 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 (Moses *et al*., 2012). Similarly, aflotoxin have emerged as a major threat to human health because a number of serious side effects such as hepatotoxicity, carcinogenecity and immuno suppression etc. are associated with them. The absence of these toxic elements in the test drug make it safe and free from serious toxic effect. The bacterial and fungal counts were although found to be present but their concentration was found to be far below the permissible limits that can not produce any toxicity. The pesticide residue were not found at all suggesting that the drug is free from contamination.

The results of the study revealed that all the safety parameters carried out on Majoon-e-Gul (MG) are within the permissible limits which indicated that the MG in powdered form is quite safe and can be used as such in the management of different ailments. It can also be used to prepare some other non sugar based dosage form such as pills, tablets and capsules etc.

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