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ORIGINAL ARTICLE

Natural Contamination of Aflatoxins in Some Crude Herbal Drugs of Agra Region

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ABSTRACT

Sixty samples of stored crude herbal drugs comprising of 10 samples each of safed musli (Chlorophytum borivilianum), satavar (Asparagus racemosus), sarpgandha (Rauvolfia serpentina), ashvagandha (Withania somnifera), mullathi (Glycyrrhiza glabara) and anantmul (Hemidesmus indicus) collected from rural traders of Agra, showed presence of aflatoxin B_1, B_2 and G_1 in quantity above the permissible limit in all the crude herbal drugs. Interestingly, aflatoxin G_2 was not detected in any of the samples screened. Aflatoxin B_1 was found to be more common in all the crude herbal drugs and its maximum concentration was recorded in samples of safed musli (350-1600 ppb) followed by sarpgandha (300-1500 ppb) and satavar (400-1350 ppb). However its minimum concentration (200-400 ppb) was noted in mullathi. Further, aflatoxin G_1 was also recorded in all crude herbal drugs but aflatoxin B_2 was only recorded in ashvagandha and sarpgandha samples in the range of 50-150 ppb and 100-250 ppb respectively.

Key Words: *Aflatoxins, crude herbal drugs, Natural Contamination*

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INTRODUCTION

The use of plants as medicines antedates history. Herbal medicines, which are also referred to as phytomedicines have played a critical role in world health for thousands of years. According to World Health Organization (W.H.O., 2013) herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredient of plants used for cure of human diseases. Over the last decade, the use of herbal medicines has expanded across the globe and gained considerable popularity. As a result of cultural and historical influences herbal medicines remain an important part of the healthcare system in India, China, and Africa (Ramawat and Goyal, 2008; Wu *et al*., 2011).

With increasing expansion in herbal medicine use globally, the quality control mechanisms surrounding the herbal medicines have become the main concern for both health authorities and the public. In the case of herbal medicines, contamination is critical to monitor. In particular, fungal/microbial contamination has been a global concern for decades. According to prior investigation (Garg, 2017) species of *Aspergillus, Penicillium, Fusarium* and *Alternaria* were found associated with crude herbal drugs of root origin. These toxigenic fungi produce mycotoxins, which are secondary metabolites, that could contaminate various plants when in the field or at any stage during the collection, handling, transportation or storage. Among all the known mycotoxins, the most toxic one is aflatoxin B_1 (AFB_1). It was classified as a group-1 carcinogen by the International

Agency for Research on Cancer due to its strong toxicity (IARC, 1993) and represents the main threat worldwide.

Thus, the present investigation was undertaken to evaluate aflatoxin contamination in stored samples of six crude herbal drugs, which include safed musli (*Chlorophytum borivilianum*), satavar (*Asparagus racemosus*), sarpgandha (*Rauvolfia serpentina*), ashvagandha (*Withania somnifera*), mullathi (*Glycyrrhiza glabara*) and anantmul (*Hemidesmus indicus*).

MATERIALS AND METHODS

Sixty samples of crude herbal drugs, comprising of 10 samples each of safed musli, satavar, sarpgandha, ashvagandha, mullathi (Liquorice) and anantmul were collected from traders of Agra region. The samples were collected in sterilized polythene bags and sealed over flame. These samples were analysed for association of moulds following dilution plate method (Graves and Hesseltine, 1966) and for natural contamination of aflatoxins following procedure outlined by Thomas, *et al*. (1975).

For extraction of aflatoxins, samples were powdered and 50g powder in each case was blended with 250 ml methanol: water (60: 40 v/v) for 2 min. at 2000 rpm. The extract was filtered through Whatman No. 1 filter paper. Then 125 ml of this filtrate was extracted with 30 ml saturated sodium chloride (NaCl) solution and 50 ml n-hexane in a 250 ml separating funnel for 2 minutes. The lower methanol layer was transferred to another separating funnel. Finally, the lower methanol layer was extracted with 40 ml chloroform. Then, the chloroform layer was drained into a flask containing 5g cupric carbonate and was allowed to settle. Chloroform was decanted through Whatman No. 1 filter paper containing anhydrous sodium sulphate. This chloroform extract was used for qualitative and quantitative detection of aflatoxins by Thin Layer Chromatography (TLC).

For TLC, silica gel-G (with 13% $CaSO_4$ as binder) was used as stationary phase. Fifty micro-litre (50 µl) of chloroform extract was spotted on TLC plates. The spotted plate was developed in the solvent system comprising toluene: isoamyl alcohol: methanol (90: 32: 2 v/v/v). After developing, the plates were air dried and were observed under long wave (360 nm) UV light for detection of mycotoxins. The spots of aflatoxins were identified by comparing with the spots of standards obtained from Sigma, U.S.A. The spots were eluted in chloroform and the quantitative estimation of the same was carried out with the help of UV-spectrophotometer (AOAC, 1984).

RESULTS AND DISCUSSION

Perusal of Table 1 clearly indicates that aflatoxin contamination is fairly common in stored commercial samples of crude herbal drugs collected from 6 tehsils viz., Khandoli, Kiraoli, Kheragarh, Fatehabad and Shamsabad of Agra district. It is interesting to note that aflatoxin B_1 (AFB_1) was found in the range of 200-1600 ppb in different herbal drug samples. Its maximum concentration was recorded in samples of safed musli (350-1600 ppb) followed by sarpgandha (300-1500 ppb) and satavar (400-1350 ppb). However, its minimum contamination (200-900 ppb) was noted in mullathi. Further, ashvagandha and anantmul samples showed aflatoxin B_1 contamination in the range of 250-1200 ppb and 250-1150 ppb respectively.

In general, contaminated samples of all crude herbal drugs revealed the presence of aflatoxin B_1 and aflatoxin G_1. Interestingly, samples of ashvagandha and sarpgandha also revealed the presence of aflatoxin B_2 in the range of 50-250 ppb in addition to aflatoxin B_1 and G_1. However, no crude drug sample showed the presence of aflatoxin G_2. The natural contamination of aflatoxins in samples of crude herbal drugs corresponded to the incidence of toxigenic fungi specially strains of *Aspergillus flavus* with them and their respective potential to produce aflatoxins in the liquid medium (Garg, 2017). It is noteworthy to mention here that concentration of aflatoxin was above the permissible limit in all the six crude herbal drugs studied and naturally occurring mixture of aflatoxins

particularly aflatoxin B_1 has been classified as class 1 human carcinogen (IARC, 1993). Further, aflatoxin contamination in food and herbal drugs is a serious cause of illness particularly liver disorders in developing countries, which have fewer safety regulations. There is urgent need to restrict the use of aflatoxin contaminated crude herbal drugs for direct consumption by human beings and effective ways for prevention and control of hazardous fungi and their mycotoxins should be worked out.

Table 1: Aflatoxin contamination in some crude herbal drugs of root origin

S. No.	Name of drug plant	No. of samples		% samples contaminated	Range of Aflatoxin contamination			
		Analysed	Conta-minated		B_1	B_2	G_1	G_2
1.	Safed musli (*Chlorophytum borivilianum*)	10	8	80	350-1600	-	150-700	-
2.	Satavar (*Asparagus racemosus*)	10	8	80	400-1350	-	100-500	-
3.	Ashvagandha (*Withania somnifera*)	10	6	60	250-1200	50-150	150-400	-
4.	Sarpgandha (*Rauvolfia serpentina*)	10	7	70	300-1500	100-250	200-500	-
5.	Mullathi (*Glycyrrhiza glabara*)	10	4	40	200-900	-	100-350	-
6.	Anantmul (*Hemidesmus indicus*)	10	5	50	250-1150	-	150-450	-

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