Mycotoxicological control on raw material and tablets of cascara sagrada (Rhamnus purshiana)

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Abstract

Among phytotherapic medicines, tablets of cascara sagrada (Rhamnus purshiana) dried bark, usually used as laxative, are commercially widespread in our market. Taking into account natural origin and/or inappropriate procedures that may allow the occurrence of toxinogenic Aspergillus flavus group, a study on susceptibility to aflatoxin contamination and natural aflatoxin incidence was performed by TLC and HPLC methods. This survey allows one to conclude that bark of Cascara Sagrada is a good substrate for the growth of A. parasiticus NRRL 2999 and for aflatoxins production. Natural aflatoxins presence was detected on 2 from 9 raw material samples. One of them (irradiated sample) had only aflatoxin B1 (10 µg/kg) and the other (pasteurized) was positive for aflatoxin B1 (19 µg/kg); G1 (6 µg/kg) and B2 (1.46 µg/kg). Only one from 10 lots of tablets analyzed was positive for aflatoxin B1 (5.42 µg/kg) and B2 (0.32 µg/kg).Therefore, adequate quality control including an aflatoxins assay must be performed to guarantee the harmlessness of natural drugs.

Introduction

Phytotherapic medicines are drugs that contain, exclusively, plant material/s and/or vegetable drug preparations as active ingredients. Marketing of these medicinal drugs has increased in the last years due to more consumers prefering natural, instead of synthetic products, believing that the natural product is a guarantee of harmlessness.

Assurance of safety, quality control and efficacy of both, medicinal plants and phytotherapic products, have become priorities in many countries. In the demand to ensure quality products, microbial status is one of the most important issue to control. All drugs for oral use must fulfill the so-called Federal International Pharmaceutical (FIP) requirements for microbial purity of non-compulsory sterile medicines (Table 1) (1). Among these specifications, only fungal enumeration was taken into account. However, this requirement is not enough to ensure microbiological security of phytotherapic drugs because there is always the possibility that inappropriate drying procedures, storage or inadequate handling may result in the growth of mycotoxin-producing fungi. Of particular concern is the contamination with aflatoxin-producing

strains from the Aspergillus flavus group (2). Aflatoxins are considered to be especially dangerous to human health due to their genotoxic and carcinogenic properties (3).

Among the phytotherapic medicines, tablets of cascara sagrada (Rhamnus purshiana) dried bark are commercially widespread in our market. Their beneficial properties include: a) its cathartic action is mild,; b) its action is limited to the large bowel; c) it is not habit forming and d) it can correct habitual constipation by restoring normal intestinal tone (4).

Cascara sagrada is a tree normally found in the west coast of USA, in a wide area from California to Washington State and British Columbia (5). Consequently, dried bark must be imported by Argentinian pharmaceutical industries for manufacturing drugs

This survey attempts to determine the susceptibility of cascara sagrada dried bark to aflatoxins contamination and to show natural aflatoxins incidence in, both, raw material and tablets marketed in Argentina.

Table 1 – Microbial quality requirements of F.I.P.

Category	Administration route --	Total Count cfu/g or ml	Enterobacteriaccae cfu/g or ml	Yeast and moulds cfu/g or ml	Not detectable
III	Oral	≥ 1000	≥ 100	≥ 100	P. aerruginosa S. aureus E. coli Salmonella

Material and Methods

Samples: Approximately, 250 g of each sample taked at random were obtained from the sole 5 pharmaceutical regulated industries that work with cascara sagrada bark to elaborate drugs. They were classified as:

- 9 from imported raw material, 7 from USA, treated with 10 Kgy gamma radiation or ethylene oxide and 2 from Italy, pasteurized
- 10 from different lots of finished products, 1 of them manufactured from a pasteurized raw material involved in this study.

Fungal counts: Decimal dilutions of all samples were made in 0.1% peptone solution and 1 ml of each was inoculated, in duplicate, into Dichloran Rose Bengal Chloramphenicol Agar (DRBC) plates. Colonies were counted after 7 days incubation at 27º (6)

Fungal Isolation and Identification: All Aspergillus spp isolated were identified using specific media and the proper guide (7).

Artificial contamination: Samples of 3 g each, after UV light treatment during 30 minutes, were moistened with sterile water (approximately 3 ml), in triplicate, inoculated with 1 ml of homogeneous heavy suspension of A. parasiticus NRRL 2999 (108 cfu/ml), and incubated at 27º C for 5, 15, 20 and 30 days. Approximately 3 g of sterile rice moistened with 50% water and inoculated with the same fungal suspension was used as positive control for toxins production (8)

Aflatoxins Analysis: Detection by TLC was used as a qualitative method for all