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Extraction of Aspergillus niger van Tieghem, an Allergenic Microfungus, and Application of Toxicity Test

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ÖZET

Allerjik Bir Mikrofungus olan Aspergillus niger van Tieghem'in Ekstraksiyonu ve Toksisite Testinin Uygulanması

Bu çalışmada Aspergillus niger van Tieghem Türkiye' de ilk defa Belgrad Ormanı toprağından izole edilmiş, ekstresi hazırlanmış ve toksik etkilerini tayin etmek için fareler üzerinde toksisite testi uygulamaları yapılmıştır. Ekstraksiyon literatürdeki metoda uygun olarak hazırlanmıştır. Bu maksatla ekstraktif olarak Coca solüsyonu, sterilizasyon için steril filtrasyon tekniği kullanılmıştır. Stok çözelti toksisite testi uygulamalarından önce 1/10 oranında seyreltilmiştir. Hazırlanan A.niger van Tieghem ekstresinin steril olduğu ve 10 fare üzerindeki deneyde toksik etkisi olmadığı bulunmuştur.

Anahtar kelimeler: Allerji, A.niger ekstresi, toksisite testi

SUMMARY

In this study, for the first time in Turkey, Aspergillus niger van Tieghem, isolated from the soil of Belgrad Forest, was extracted and some toxicity tests were carried out in order to determine its toxic effects on mice. The method of extraction complied with the one described in the literature. During the process Coca's solution was used as an extractive agent. Sterile filtration technique was employed in the sterilization process. The stock solution was diluted to 1/10 of its concentration before the application of toxicity tests. It was made certain that the A.niger van Tieghem extract was sterile and that, as the experiments made on 10 mice proved, it was not toxic.

Key words: Allergy, A.niger extract, toxicity test

INTRODUCTION

Hypersensitivity or allergy is a matter of great importance to physicians. In 1906, von Pirquet used the term allergy in the sense of a reaction other than the normal one, and he described the state as an ability in the body which develops as a result of a first contact with organic or inorganic substances and which, at the second contact, causes a reaction of different characteristic, intensity and timing. Today our understanding of allergy allows us to define it as a specific hypersensitivity against an antigen damaging tissues. Allergen is the name given to antigens or haptens causing allergy. Allergens are found in various forms ranging from foreign proteins to simple substances as quinine and chrome. Freezing and exposure to certain rays may also result in allergies (1).

In the usual method for extracting allergenic substances to be used in intra-cutaneous or intra-dermal tests, the process is as follows: sifting, extraction, clarification, sterilization, sterility test and standardization (2). In the process of extraction Coca's solution is used.

Stock should be sterile and be free from pyrogen. In the process of filtration, a membrane filter with a pore width of 0.80, 0.45 and 0.20 µm is used. It is suggested that the pH of the extraction should be 8.2.

Contact allergy often stems from proteins (antigen, allergen). Allergies are divided into 4 groups: Type I; early or immediate allergy, Type II; allergy damaging cells (allergy to medicine), Type III; allergy caused by antigen - antibody compounds and Type IV; late reaction allergy, contact allergy or contact dermatitis. The difference between type I and type IV is that the former has a high molecular structure whereas the latter has a low molecular weight of 100-1000 (2). Alternaria, Aspergillus, Cladosporium, Fusarium, Helminthosporium, Mucor, Penicillium, Phoma, Rhizopus and Trichothecium are various microfungi, some of which are found in soil and known to cause allergy, and so is A.niger van Tieghem (3). In this study, results obtained from a research made on A.niger van Tieghem, its extraction and toxicological applications have been presented.

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MATERIALS AND METHODS

a)Soil sample and isolation and identification of A.niger

In March 2000, a soil profile in Belgrad forest was opened and the surface was cleaned up vertically. The sample was then taken from a depth of 10 cm in aseptic conditions. Afterwards it was mixed and left to dry in room temperature. In the process of isolating microfungi from the soil sample, "Soil Dilution Plate Method" was employed (4). The dried sample was added into some sterile distilled water to form a 1/10 suspension. The suspension was mixed for 30 minutes in a mechanical mixer (5). More sterile distilled water was added to the liquid to form 1/100, 1/1000 and 1/10000 dilutions. Of these the most apposite for use were 1/1000 and 1/10000 ones, the latter of which was preferred in our study (6). Before the organic matters and soil particles were deposited (7), 1 ml of the latter suspension was cultivated on a medium of Peptone Dextrose Agar (8,9) with a sterile pipette(10). In order to prevent supressed growing of bacteria and Actinomycetes from producing 30 mg/l streptomycin was added into the medium along with the same amount of rose bengal aimed at limiting the size of colonies (8). Of the microfungi colonies which formed after an incubation period of 10 days at 25 °C, Aspergillus genus was isolated and cultivated in the Czapex Dox Agar medium (11). Following another ten-day incubation period at 25 °C, the preparation was dyed with picric acid and identified by means of lactophenol solution (12).

b)Coca's solution

Coca's solution was used so that the agent in A.niger would pass into the extracted material. Coca's solution consists of NaCl, phenol, $NaHCO_3$ and distilled water (2).

c)Extraction

9 ml of Coca's solution was added into 1g of A.niger. The mixture was mixed for 24 hours at 4 °C in a magnetic mixer. It was then centrifuged for 10 minutes at 2500 rpm. Following this process the extract was centrifuged twice more for the same period of time (2).

d)Filtration

The extract was first filtered through a rough filter wetted with Coca's solution. Later on it was filtered through S&S black bandaged paper and sterilised in a laminar cabinet using the Sterile Filtration Technique. At this stage the extract was filtered in Sartorius sterile filtration injector through membrane filters of 0.80 µm, 0.45 µm and 0.20 µm pore diameters.

e)The dilution of pure extract before controls

Extracts which belong to a particular species and which are obtained by means of sterile filtration are called pure extracts (2). Aytuğ et al. (2) have stated that extracts should not be used in diagnosis and treatment of allergy, in sterility and toxicity controls and in skin tests unless diluted as much as necessary. This is why the pure extract used in this study was diluted.

Special Diluent Solution I:

0.9 % NaCl + 0.5 % phenol + distilled water→1000 ml

Special Diluent Solution II:

Special Diluent Solution I + glycerine (50:50)

For Sterility and Toxicity Test:

Special Diluent Solution I + special Diluent Solution II + pure extract (9:1)

For sterility and toxicity tests 1% extract containing 5 % glycerine was used.

f) Sterility Test**

For these tests to be conducted anaerobe and aerobe media were used. 2-3 drops of extract in Thioglycollatte were examined for 14 days at 35 °C and in Sabouraud Dextrose Agar for the same period of time at 25 °C(2).

g) Toxicity Test ***

Laboratory animals used: 10 mice (Balb/c strain)

Weight of mice:

1st experiment group: 22.9, 22.9, 23.2, 24.5, 22.5 g (mean 23.2 g)

1st control group: 22.9, 22.9, 23.2, 24.5, 22.5 g (me-

an 23.2 g)

2nd experiment group: 20.9, 30.5, 29, 29.4, 27.1g (mean 27.38g)

2nd control group: 20.9, 30.5, 29, 29.4, 27.1g (mean 27.38g)

Each mouse was injected 0.5 ml diluted extract subcutaneously in their abdomen. Mice were then followed and their diet was not changed. On the 8th day they were reweighed(2).

Table 1 a. First and last weight of the 5 mice subjected to toxicity test in the 1st experiment group

	1st group	
Weight of mice	Total weight	Weight of mice
Weight of mice before injection	116 g	23.2 g
Weight of mice 8 days after the injection of A. niger exract	126.9 g	25.38 g

Table 1 b. First and last weight of the 5 mice in the 1st control group

	1st group	
Weight of mice	Total weight	Weight of mice
Weight of mice before controls	116 g	23.2 g
Weight of mice after 8 days	126.9 g	25.38 g

Table 2 a. First and last weight of the 5 mice subjected to toxicity test in the 2nd experiment group

	2nd group	
Weight of mice	Total weight	Weight of mice
Weight of mice before injection	136.9 g	27.38 g
Weight of mice 8 days after the injection of A. niger exract	146 g	29.2 g

Table 2 b. First and last weight of the 5 mice in the 2nd control group

	2nd group	
Weight of mice	Total weight	Weight of mice
Weight of mice before controls	136.9 g	27.38 g
Weight of mice after 8 days	146 g	29.2 g

(**) Sterily tests were carried out in GATA Haydarpaşa Educational Hospital, Microbiology Department, Istanbul, Turkey

(***) Toxicoligal tests were carried out in Marmara University , Medical Faculty Experimental Research and Animal Laboratory, Istanbul, Turkey

RESULTS AND DISCUSSION

It is in accordance with the literature that A.niger is isolated from soil and that it is allergenic (3). As evidenced by the reproduction of no single microorganism in the sterility test, the extract was sterile. That no mortalities were observed among the 10 mice during the toxicity test is enough evidence that the extract was not toxic. 8 days after the injection of A.niger, it was observed that the weight of the 5 mice in the 1st experiment group increased by 10.9 g totally (mean 2.18 g), (Table 1a). The same increment was observed in the 5 mice in the 1st control group after the same period of time (Table1b). Likewise, 8 days after the injection of A.niger the 5 mice in the 2nd experiment group increased 9.1 g totally (mean 1.82 g) in weight (Table 2a). The same increment in weight was observed also in the 2nd control group (Table 2b), which is in accordance with the literature (2,13).

Aytuğ (14), Aytuğ et al. (2), Said El Shami and Merrett (15), Çolakoğlu (16,17,18) and Ada (13) have significant studies on allergy, and they commonly seem to share the view that, although harmful as an allergen.

A.niger is a microfungus of wide use in industrial microbiology. Fungus metabolites are one of the fields where fungi are mostly used. In order to enable it to reproduce a metabolite of its kind, fungus is kept under optimum conditions. The substance produced by the organism is then isolated. According to Pekin (19), A.niger species is an important source in industrial microbiology in producing such enzymes as acid resistant amylase, glycoamylase, invertase, pectinase, protease, glucose oxidase, naringynase, lactase; and in producing gluconic and citric acids from organic acids (20).

In view of the results obtained from this study, the author believes that it will be beneficial for the economy of the nation if extracts from microfungi of Turkish origin are made use of in medical and industrial microbiology.

This is a first study of its kind in Turkey in the field of mycology aimed at producing extracts. We, therefore, hope that it will be a guide for other researchers in both the process of extracting and its toxicological applications.

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