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Evaluation the immune suppressive effect of aflatoxin B1 in mice
التأثيرات المناعية الكابحة للـ aflatoxin B1 في الفئران

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Abstract:

Aflatoxin B1 cause immune suppression especially in cellular immunity and decrease the number of T and B cells .The percentage of both CD4+ and CD8+ Tcells which are, in general, used as markers for helper and cytotoxic T cells ,are affected by aflatoxin B1 and showed lower percentage when injected with aflatoxin B1 four successive doses in mice for 16 days (CD4+Tcell =224.25 , CD8+Tcell =177) and eight successive doses for 32 days (CD4+Tcell =159 , CD8+Tcell =144) when compared with the untreated mice (control group) (CD4+Tcell =361 , CD8+Tcell=237.5)for 16 days and (CD4+Tcell =338.33 , CD8+Tcell=257)for 32 days.

الخلاصة:

يسبب aflatoxin B1 تثبيط الجهاز المناعي سيما المناعه الخلويه ويقلل عدد الخلايا Tو B. تتأثر خلايا T بنوعيها CD4+و CD8+ التي تستخدم كدلائل للخلاياTالمساعده والسميه على التوالي ، بوجود aflatoxin B1 ،عند حقن aflatoxin B1 اربعة جرع ناجحة في الفئران لمده 16 يوم وحساب خلايا T CD4+ و CD8+ اظهرت النتائج انخفاض نسبة خلايا Tالمساعده و السمية (خلايا T CD4+= 224.25 ، خلايا T CD8+= 177) عند مقارنتها مع الفئران غير العامله ب aflatoxin B1 (مجموعه السيطره) (خلايا T CD4+= 361 ، خلايا T CD8+= 237.5) وعند استمرار الحقن بaflatoxin B1 بثمان جرع ناجحة لمده 32 يوم وجد هناك زياده في النقصان (خلايا T CD4+= 159 ، خلايا T CD8+= 144) عند مقارنتها مع المجموعه غير المعامله (خلايا T CD4+= 338.33 ، خلايا T CD8+= 257).

Introduction

Numerous studies conducted in animals , and human cell cultures have shown that aflatoxin exposure can suppress immune function especially cell-mediated immune responses [1, 2]. More specifically, these studies on the immunotoxic effect of aflatoxin have shown that exposure to aflatoxin decreased T or B lymphocyte activity [3, 4] modified synthesis of inflammatory cytokines [5], suppressed NK cell mediated cytolysis [6], decreased resistance to infectious diseases [2, 7], induced reactivation of chronic infection [8], decreased immunity to vaccination [9, 5], and impaired immune function in developing animals [10]. Aflatoxin B1, the most toxic of the aflatoxins, is the most potent naturally occurring known chemical hepato carcinogen. Specific P450 enzymes in the liver metabolize aflatoxin into a reactive oxygen species (aflatoxin-8,9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer .

This study aimed to evaluate the immune suppressive effect of aflatoxin B1 in mice.

Materials and Methods

1- Preparation of Aflatoxin B1 Solution

Aflatoxin B1 tube (sigma) contain 10 mg of lyophilized toxin was dissolved in 2 ml of acetonitral and then had been diluted to 100 ppm and kept in the dark conditions at -20°C until use [11].

2-Laboratory Animals:

Twenty healthy male mice were obtained from national center for maintenance of drugs . Animals age was ranged between 6-8 weeks, their weight was about 25-30g. Animals were placed in small plastic cages, every cage size was about 29×12.5×11.5 cm, and each cage contains 5 male mice.
mice were randomly and equally divided into two groups ,first group injected with 0.1 of 100 ppm Aflatoxin B1 every 4 days ,half of the animals sacrificed after 16 days (having 4 doses) and the rest were sacrificed after 32 days (having 8 doses).
Second group was kept only on the basal diet (control), half were sacrificed after 16 days and the rest were sacrificed after 32 days with heart puncture .
Mice were anesthetized by chloroform for gathering blood samples using insulin syringe. Blood was collected from the mice by heart puncture after 16 and 32 days, sera was separated by centrifuging at 5000 rpm for 10 min.,then we used CD4 and CD8 Bio Assay ELISA Kits, following kits procedure and instructions

3-Immunological examination

calculation of CD4 :

1- A microtiter plate , a wash buffer (10 X) , a blocking / diluent buffer were brought to room temperature (18-25°C)

2- The wash buffer (10 X) was diluted 1:10 with ddH2O for use

3- The microtiter plate was removed from the shielded bag ,then 200 µl of dilute wash buffer were added to each well and left at Room temperature until being ready for the next step

4- Several dilutions (minimum 200 µl)of standard 1000ng/ml were prepared in a Blocking / Diluent Buffer in Eppendorf tubes – labeled accordingly :500ng/ml to 0.5 ng/ml in 2-fold serial dilution .

5- Test samples were prepared using a blocking /diluent buffer in 100 ng/ml range.

6- The well's contents were emptied and pated dry ,then a strip well holder was set up on a grid.

7- One hundred µl of positive reference (standard) and test sample were pipetted into wells. The plate was covered and left at room temperature for 1 hour .

8- The well's contents were emptied and washed 3 times with 1X wash buffer (300 µl /well).Left last wash until ready to add a detector reagent .

9- Then diluted CD4 Ab (HRP) 1:10 in a blocking /diluent buffer .Added 100 µl diluted CD4 Ab (HRP) to each well . Plate Left at RT for 1 hour , washing was then repeated 3 times and left last wash in well until ready to add substrate.

10-The contents of wells were emptied and added 100 µl of TMB substrate to each well , color (Blue) was developed over 5-30 min at RT.

11-The color development was stopped after 10 minutes by adding 50 µl of stop solution to each well ;color changed to yellow . Plate was read at 450 nm as soon as possible .

calculation of CD8 :

1. The number of microwell strips required were determined and washed twice with Wash Buffer.
2. One hundred microliter of Assay Buffer (1x) was added , in duplicate, to all standard wells. Pipetted 100 µl to prepare standard into the first wells and create standard dilutions by transferring 100 µl from well to well ; then 100 µl from the last wells was discarded
3. One hundred microliters of Assay Buffer (1x)was addded, in duplicate, to the blank wells.
4. Fifty microliters of Assay Buffer (1x)was added to sample wells.
5. Then 50 µl of the serum sample was added in duplicate, to designated sample wells.
6. Microwell strips were covered and incubated for 2 hours at room temperature(18°C to 25°C).
7. Detection Antibody was prepared .
8. Microwell strips were emptied and washed 3 times with Wash Buffer. Then 100 µl Detection Antibody was added to all wells , covered and incubated for1 hour at room temperature (18° to 25°C).
9. Microwell strips were emptied and washed 3 times with Wash Buffer , then 100 µl of diluted anti-rabbit-IgG-HRP was added to all wells , which were covered and incubated for 1 hour at room temperature (18° to 25°C), emptied and washed 3 times with Wash Buffer
10. Then 100 µl of TMB Substrate Solutionwas added to all wells , which were Incubated for about 10 minutes at room temperature (18° to 25°C), then added 100 µl of Stop Solution to all wells.
11. Blank microwell reader and measure color intensity at 450 nm.

Reading of the results:

The sample results were calculated by interpolation from a calibrator curve that is performed as that of the sample. The curve has been drawn; the concentration of the calibrators (standard) was plotted on the horizontal axis and on the vertical axis the corresponding absorbance. Then the absorbance for each sample was located on the vertical axis and read off the corresponding sample concentration on the horizontal axis.

Results and Discussion

The design of the dosing AFB_1 to mice was originally conceived to certain aspects of risk assessment with regard to cellular immunity such as dose and duration of exposure. it was known that AFB_1 affects immune function in various animal species [3]. This aspect of AFB_1 toxicity could be important also for risk assessment extrapolations, if the immune system, via the inflammatory process or other mechanisms, is involved in carcinogenicity [11]. This study, investigate the effects of AFB_1 on the main cellular immunity by esstimation the level of CD4+ and CD8+ T cells after injected mice with AFB_1 for 16 days,32 days and comparison the result with control group(without injected with Aflatoxin B1) **.** The result clarified that there was a significant difference between the CD4+ T lymphocyte, CD8+ T lymphocyte percentages in mice injected with Aflatoxin B1 and those of the control group. CD4+T lymphocyte and CD8+ T lymphocyte

percentages were significant lower(CD4+ T cell =224.25 , CD8+Tcell =177)in the injected mice for 16 days when compared with control (CD4+Tcell=361,CD8+Tcell= 237.5) as shown (Fig. 1).. While it was found much reduction in the level of CD4+ T lymphocyte and CD8+ T lymphocyte percentages(CD4+Tcell =159 , CD8+Tcell =144) in mice injected with Aflatoxin B1 for 32 days than in the control group CD4+Tcell =338.33 , CD8+Tcell=257)as shown (.Fig. 2).

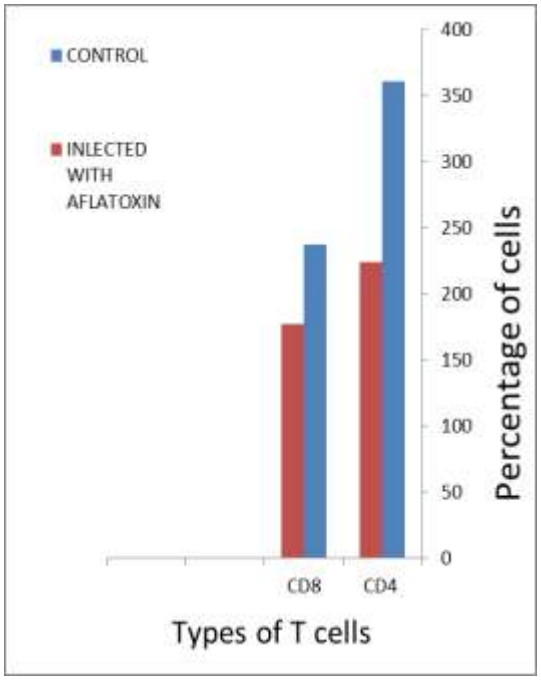

Figure 1 :The percentage of T cells in mice treated with aflatoxin B1 for 16 days.

Butler reported that there was "slight" inflammatory responses in rat due to AFB_1 induced injury in the liver and Kubena *et al.*,[9] mentioned that absolute counts of CD4+, CD8+ cells and total lymphocytes were decreased very significantly during exposure to AFB_1. As in our study, other investigators have also reported lower absolute counts of CD4, CD8 cells and total lymphocytes during treating animals with AFB_1[14, 15].

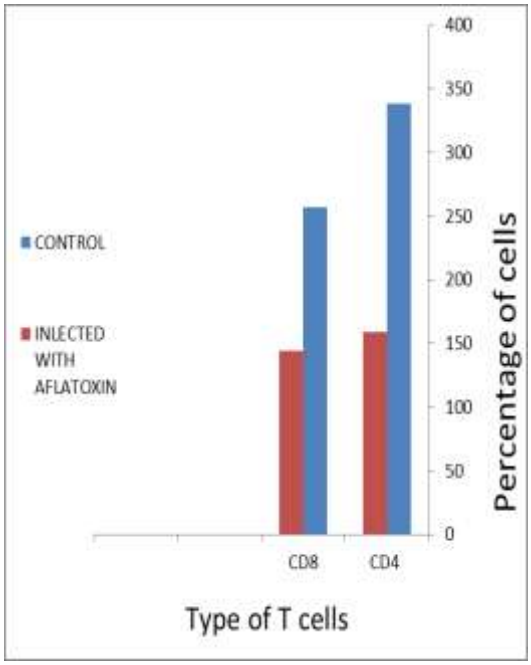

Figure 2 :The percentage of T cells in mice treated with aflatoxin B1 for 32 days.

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