Investigation of Aflatoxin B1 in blood of diabetes mellitus type 2 patients

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Abstract
The aim of study was investigation of aflatoxin B1 in blood serum of patients with diabetes type samples were collected from Al-Hussian hospital, Karbala province. The number of samples were (86) samples (42) samples of which were for patients with diabetes disease and (44) samples were for healthy people. The results showed that 16 (38.1%) out 42 samples that collected from patients with diabetes types of contamination with aflatoxin B1. While 13 (29.5%) sample that collected from healthy persons were contamination with aflatoxi B1 with no significant difference between them. Also, the number at blood serum collected from females and males' patients with diabetes types and contamination with AFB1was 17 (19.7%) and 12 (13.9%) respectively. The results illustrated the AFB1 concentration in blood serum of females and males' patients with diabetes was 1.343 ng/ml and 0.684 ng/ml respectively with significant difference between them while the concentration of toxin in blood serum of females and males was 0.133 ng/ml and 0.135 ng/ml respectively with no significant difference between them. Result: The measurement coefficient r appears there is correlation between gender and precense of AFB1 in blood serum (r =0.974 p value 0.324) it means the females more sensitive to AFB1 compare with males. Conclusion: Aflatoxin B1 contamination of blood serum patients with diabetes type 2 also AFB1 contamination blood serum of healthy persons but with low concentration compare the diabetic patients. Females more sensitive to Aflatoxin B1 compare with males. Diabetic disease led to increase the Aflatoxin B1 level in blood serum and this may be due to the ability of the AFB1 to cause tissue damage in the kidney tubules which lead to poor efficiency of the kidney function in excreting the AFB1.
Keywords: _ Aflatoxin B1, Diabetes mellitus type 2.

1. Introduction

Mycotoxins are chemicals compounds that develop by filamentous fungi that disrupting human and animals health by mycotoxicosis that different from tradition mushroom poisoning, These fungi called" toxigenic fungi", All of those species are Deuteromycetes (asexuell) some of which have we known Ascomycetes (sexuall) stage. Mycotoxin are secondary metabolite of the fungi concerned, these are compounds that developed after one or more nutrient become limiting [1]. the production of mycotoxin are absolutely determined by the presence of situation that support the growth of the fungi concerned, under environmental conditions. Different fungi species are favored to cause disease on the crops in the farm, while saprophytes tend to grow on the stored crops or sometimes grow on other materials.

The studies of toxigenic fungi reveal that they developing many compounds often form different biological functions the environmental conditions impact in the produceing of these compounds has been a fertile major of study in the recent because mycotoxin influence was known [2]

When Penicillin discovered and other fungal derived antibiotic, the scientists considered that these compounds active in nature. In the post war period, secondary metabolites were characterized as everything from waste product the consequence of "displacement activities" of the fungi. these compounds are significant as virulence factor and as mediators of interference competition with enzyme lead to inhibition activity of it's [4]

2. Materials and Method

The present work included a case_control study from November 2021 till March 2022, samples were collected from patients attending Al Hussein medical hospital the sociodemographic aspects of the patients were collected through the self reported techniques (study questionariae) including gender and diabetes mellitus type 2 patients.

For the relationship purpose patients were divided certain etiology of diabetes mellitus type 2 patients' group were compared to group who don't have diseases (appearantlly healthy) as a control subject

A total of 86 subjects were studied 42 (21 male and 21 female) of them patients with diabetes type 2 44 (22 male and 22 female) healthy people do not suffer from diabetes.

The study protocol was approved by the ethical research committee, college of applied medical science, university of Karbala. Groups of this study including eight groups as the following table.

Taken 1.5 ml from each blood serum samples by

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streile micropipette and transported to streile test tube and added to each one sample 50µl from Protinase k solution and left react for 10 minutes. After that the mixture was exposed to centerifugation for 15 minutes at 2500 rpm. Then from each sample the filtrate was taken and the preciptate was neglacted. Then 1mL chloroform was added to each filtrate and shake vigorously in the electeric shaker device, where it formed tow (blood serum layer and chloroform layer). Chloroform layer was separated by sparating funuel and put in sterile other glasses tube and let to evaporate.

A thin layer chromatography plate was coated with silica gel. Dimension of (20×10) cm was used after activated it in electeric oven at 120C° before using a light straight line was made at a distance of 1.5 cm from the bottom and top of the plates the bottom line was used for loading samples and the top line was used for numbering. The mobile phase used to separate AFB1 was chloroform 95: methanol 5. Stander of AFB1 (10µl) was added as a spot on TLC plate by capillary tube then 20µl from each extracted sample were added on the plate with a distance of 2cm between samples after that these spots were left to dry in the room temperature. The plate finally were settled in the separation tank which containing mobile phase.

Then thin layer plate was left in the tank until the mobile phase reached 2cm from upper edge of the plate . After that TLC plate was removed from the tank and left dry in the room temperature.

Then plate was examined under UV light (365nm) and compare the color and relative flow (RF) of extracted sample with stander toxin.

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Analysis of AFB1 by high performance liquid chromatography the separation carried out by liquid chromatography model SYKAM (Germany) by Fx 10 A_flouerscent detector (Liu et al., 2012).

Taken 0.5 mg sample powder was dissolved in 30mL 0.1%. Trichloro acetic acid then agitated in ultrasonic bath for 5 minutes. The standard aflatoxin was separated on fast liquid chromatographic. Next the extract were filtered on dispoable sepak filtred paper 0.5 milpore to remove the fibers.

Average of duplicate reading of samples were performed measurement were prepared using the average blank _corrected for each sample standard AFB1 concentration in ng/mL to detemine the concentration of each unknown sample.

3. Result and Discussion

The result showed 16 (38.1%) out 42 Samples of Serum Collected from patients with type -2 diabetes contamination with AFB1 . While 13 (29.5%) Samples From nu Samples Collected

From Healthy persons. (Control with Aflatoxin B1 were Contamination). Also the result appearance that 26(61.9%) Collected From patients with diabetes not Contaminated with AFlafoxing B1. Addition 31 Samples collected from healthy person group were not contamination with toxin (Table 1) statistical analysis didn't Showed significant difference between the umber Sample that Contamination with AFB1 and the number samples that Contamination An AFB1 which tis Collection from healthy person. group. This study showed the number of blood Serum collected from male and female patients contamination With AFBI was 17 (19.7%) and 12 (13.9%) respectively withou. significant difference between them

Table(1) The number and percentage of Samples that collected from patients and healthy . that borne of AFB1

Case	Without AFB1	With AFB1	Total
Healthy	31 (70. 5%)	13 (29. (5%	44
Patients	26 (61. 9%)	16 (38. 1%)	42
Total	57(60. 30%)	24 (33.7%)	86 (100%)
X^2 Calculate = 2.48 X^2 table = 3.84			

While the number of the blood serum that Collected from females and males and Without AFB1 was 27 (31. 39%) and 30 (34.88%) respectively (Table2).

Table (2) The number and percentage of Serum Samples. that collected from males and female . borne AFB1

Gender	Without AFB1	With AFB1	Total
Males	30 (34%)	12(13. 9%)	42
Females	27(31. 39%)	17(19. 7%)	44
Total	57(66. 3%)	29 (33. 37%)	86(100%)
X^2 Caculate = 0.87 X^2 table (0.05) = 3.84			

This result agreement with some studies that have carried out [5] showed that the investigated population were exposed to AFB1 was detected in 100% of uncertain chronic kidney disease (CKD) patients and 24%, 20% in certain CKD patients and healthy.

[6] found 22 out 36 Sample (61. 1%) of blood collected from persons were contain patulin by using Thin layer chromatography. The percentage of blood Samples that collections from females and

males was 54. 5% and 95. 5% respectively.
Also others study illustrated the percentage of blood serum Sample that collected from Patients (with nephropathy) and Contamination With Ohratoxin A was 90% While the percentage of the blood serum that Collected from healthy group and contamination with OTA was 3% With significant difference between them.
As well as the percentage of males. and females blood serum Contamination With mycotoxin (OTA) was 48.8% and (51.1%) respectively with no significant difference between them [7]
The results of this study showed that blood samples collected from males and female's patients with diabetes type_2 the AFB1 concentration reached to 1.343 ng/mL with significant difference from other group. Also the Samples that collected from males patients with diabetes AFB1 was 0. 145 ng/mL with significant difference from AFB1 Concentration in blood serum of Healthy group (Table-3)
This result approched with [5] result who found the concentration ranges of AFB1 in serum samples were (0.68-8.33) ng/mL for uncertain CKD patients (1.21-5.6) ng/mL for certain CKD patients and (0.11-1.30) ng/mL for healthy control.

Table (3) AFB1 Concentration (ng/ML) in blood Serum of group 3 Sindy

group	Statistical Notation	mean	SD±
Healthy, female	a	0.133	0.0035
Healthy, male	a	0.135	0.0033
Patients, males	b	0.684	0.7458
patients, females	c	1.343	0.299

Number with difference letters that there are significant difference between them at p< 0.05.

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