Effect of diabetes mellitus type 2 and Aflatoxin B1 in functional kidney and liver

Ghada M. Kadhu[1*], Sami A. R. AL_Jumaili[2], Huda A. R. AL_Hashemi[3]

[1,2]Department of Clinical Laboratories College of Applied Medical Sciences, University of Karbala, Iraq.

[3]Department of Environmental health College of Applied Medical Sciences, University of Karbala, Iraq.

Email: ghada.m@s.uokerbala.edu.iq

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 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. Measurement coefficient (r) appearance there is correlation between gender and presence of AFB1 in blood serum (r=0.974 p value 0.324) it mean the females more sensitive to AFB1 compare with male group. Males patients with diabetes and presence of AFB1 in blood serum lead to increased urea level to 42 mg/ml (abnormal level) compare (34) mg/dl in blood serum of male health. Also critinine level increased in blood serum of male patients with diabetes and born AFB1 to 1.88 mg/dl. Also uric acid level raised to 8.3 mg/dl in blood serum of male patients with diabet and born AFB1 compare with its level in male healthy without AFB1 was 5.87 mg/dl on the other hand alkaline phosphate (ALP) increased to abnormal level in (blood serum of patients male without Aflatoxin B1) group and (female patients without Aflatoxin B1) group reached to 145.8 U/L and 147.1 U/L respectively. Also alanine amino transferase (ALT) parameter raised to (18.15 and 15.08) U/L in blood serum of males patients with diabetes with Aflatoxin B1 group and males patients with diabetes without Aflatoxin B1 group respectively. The result of measurement of AST in blood serum of males and females patients with diabetes type 2 without Aflatoxin B1 raised to 53.38 U/L and 49.82 U/L respectively.

Keywords: Uric acid, Creatinine, Urea, ALT, AST, ALP,Diabetes mellitus type 2.

1. Introduction

Aflatoxins are cancerous secondary metabolites produce primarily by Aspergillus flavus and Aspergillus parasiticus in agricultural food stuff such as peant, maize, cereals, and animal feed. Aflatoxins is produced at temperature of 12_40 C° and requires 3_18 % moisture. Six out of 18 different types of aflatoxins that have been identified are considered important and are designated as B1, B2, G1, G2, M1 and M2. These aflatoxins groups exhibit molecular differences.

Aflatoxin B1 is the most common of the most widespread in the world and accounts for 75 % of all aflatoxins contaminated of food and feed. Aflatoxins are highly liposoluble compounds

Takes a high amount of aflatoxin B1 in short time lead to acute toxicity. Most common signs and symptoms are vomiting, Abdominal pain, Edema, Nausea, Itching and death.

The contamination of foods with aflatoxin B1 can cause serious consequences in human health [9]

Type 2 diabetes mellitus (T2DM) is one of the major global diseases, however it's alawyes linked with obesity. Patients with diabetes mellitus are exposed to serious complications such as (microvascular and nephropathy) and macrovascular complications such as (cardiovascular and comorbidities) owing to hyperglycemia and individuals condition of Insulin resistance metabolic syndrome occur [10]

2. 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

(Table 1) male and 22 female) healthy people do not suffer from diabetes. Table 1 group of this study		
No group	Groups	Characteristics Groups
	M,D-2,TX	male, diabetes type 2 with AFB1
	M, D-2, NT	male, diabetes type 2 with AFB1 toxin
	M, H, TX	male, healthy with AFB1 toxin
	M, H, NT	male, healthy without AFB1 toxin
	F, D-2, TX	female, diabetes type 2 with AFB1 toxin
	F, D-2, NT	female, diabetes type 2 without AFB1 toxin
	F, H, TX	female, healthy with AFB1 toxin
	F, H, NT	female, healthy without AFB1 toxin

The study consist of 42 patients with diabetes mellitus type 2 who were selected from Al Hussein medical hospital in the Karabla governorate. A questionariae was applied in order to identify important sociodemographic characteristics. Gender health status family history of diabetes mellitus and diatery habit.
Generly patients who reported congential disease in kidney and liver,, Diabetes mellitus type 1, pregnant women, children.
Control group of an appearantlly healthy 44 subject (22 male and 22 female) were chosen from well know volunteers that had no history of diabetes mellitus type 2 the percentage of female and male adult individuals were equal to patients. Demographic Information of the volunteers was also collected through the self reported techniques [student questionariae]
Measurment of qualitative of serum AFB1 was carried out by thin layer chromatography.
Protinase k solution was prepared according to instruction of the Korean junaid Company. It was prepared by adding 1.1ml streile distelled water to 22 mg of protinase powder
Preparation of AFB1 stander by disolved 500 microgram from AFB1 in two ml of chloroform become the AFB1 concentration is 250µl /ml.
Taken 1.5 ml from each blood serum samples by 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.
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.

3. Result

The results of this study showed that blood samples collected from males and females 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_1)

This result approched with [1] result which 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 (2) 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.

The study have carried out found that mycotoxin (OTA) in blood serum of 82 person with 3.5_6.8 ng/mL[2]

The incidence of positive values for AFB1 in blood was over 50% win respect to the levels found . The average where similar to those reported in the Counteries except in San Vicente de Tagua - Tagua where the woman's group presented values. higher than other reports [3] the study have appeared that Concentration OTA in blood serum of female and male patients, with nephropathy was (7.015 and 7.071) ng/ mL respectively While it was in both healthy females and males was (0-1, 0.009) ng/mL respectively[4]

The increase levels of AFB1 in blood serum of females and males patients with diabetic type_2 belong to the diabetic disease which it's cause asignificant indication of kidney impairment and toxicity. Renal function decline to about 25-50% lead to less the AFB1 excretion [5].

Increased levels of AFB1 in blood serum effected in functional of liver and kidney [3]

The result Showed that there is correlation between gender and presence of AFB1 in blood serum (r = 0.974 P= 0.324) – It mean that The females more sensitive to AFB 1 compare with males group

Also there is correlation between healthy status and presence AFB1 toxin in blood the value of coefficient (r) was 0.703 and p value equal 0.402 (Table _3) .It mean that AFB1 concentration in blood serum of patients with diabetes group highest compare of it's concentration in blood serum of healthy group.

The result approched with study that found correlation between presence of AFBI and liver disease [6]

The study have proven that statically significant correlation observed between AFB1 level in blood serum and infected with chronic kidney disease (CKD). [1] some study have found relation Ship between dose of AFB1 and grain. of infants in Gambia, Benin and Togo. [8]

Table (3) Estimation of correlation coefficient (r)

Type of Correlation	Coefficient (r)	P. Value
Gender ×blood toxin (AFB 1)	0.974	0.324
Health Status X blood toxin (AFB 1)	0.703	0.402

Measurment of some blood biochemical levels of patients with diabetes mellitus type 2 patients group and healthy groups

4. Material and Methods

The work was conducted in labs of Al_Hussein Medical hospital.

The urea assay is a modification of torly enzyme produce. The test is a kinetic assay in which the intial role of of the reaction is linear for limited period of time. Urea in the presence of glutamate dehydrogenase (GLDH) the formed ammonium ion react with alpha ketogluterate and NADH to form glutamate and NAD measured at 340 nm, NADH oxidation in unit time is proportion to the urea

concentration in the sample.
Creatinine react in Alkaline environment with picric acid forming salt of yellow orange color. The intensity of of the color that develop in the specific time interval is proportion to the amount of Creatinine in the sample
Unicase transforms uric acid into allantion with formation of hydrogen peroxide (POD) reaction with 4 aminoantipyrine and 3 hydro 2.AStriiodobenzoic acid to produce color complex which it's intensity is directly proportion to the uric acid con entration in the sample.
The present of alpha ketogluterate alanine is converted into pyruvate and glutamate by ALT/GPT in the sample in the presence of NADH and lactate dehydrogenase pyruvate is trancformed Into lactate and NAD.
NADH Oxidation in time unit was measured at 340 nm is proportional to the concentration in the sample.
In the presence of alpha ketoglutamate AST/GOT in sample transforms Aspartate into oxaloacetate and glutamate in the presence of NADH and malate dehydrogenase. Oxaloacetate is converted into malate and NAD.
Consuming of NADH per unit of time measured at 340 nm is proportional o the concentration of AST /GOT in the sample.
Alkaline phosphate ALP catalyses the hydrolysis of p_natrophenyl phosphate at PH 10.N liberating p_natrophenyl and phosphate. The rate of p_natrophenyl formation measure photoelectrically is proportion to the catlytic concentration of Alkaline phosphate percent in the sample.
Anova table and Duncan test were applied using for determine the statical significance of data p-values of under 0.05 was considered statically significant.
The correlation were done between:
Gender X blood toxin (AFB1).
Health status X blood toxin (AFB1).

5. 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_4) 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.
AFB1

Table(4) The number and percentage of Samples that collected from patients and healthy . that borne of

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%)
X2 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 (5) 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 study that have 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.[1] some studies 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. [11]
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 [4]

The results of this study showed that blood samples collected from males and females 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_6)

This result approched with result which 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. [1]

Table (6) AFB1 Concentration (ng/ML) in blood Serum of group 3 Sindy

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Number with difference letters that there are significant difference between them at p< 0.05 some report have mentioned that mycotoxin (OTA) in blood serum of 82 person with 3.5_6.8 ng/mL [2]

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Some researchers showed that Concentration OTA in blood serum of female and male patients, with nephropathy was (7.015 and 7.071) ng/ mL respectively While it was in both healthy females and males was (0-1, 0.009) ng/mL respectively [4]

The increase levels of AFB1 in blood serum of females and males patients with diabetic type_2 belong to the diabetic disease which it's cause asignificant indication of kidney impairment and toxicity. Renal function decline to about 25-50% lead to less the AFB1 [5].

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Also there is correlation between healthy status and presence AFB1 toxin in blood the value of coefficient (r) was 0.703 and p value equal 0.402 (Table_3) .It mean that AFB1 concentration in blood serum of patients with diabetes group highest compare of it's concentration in blood serum of healthy group.

The result approched with study of Scientific researcher [6] Who found correlation between presence of AFBI and liver disease

Study have mentioned that statically significant correlation observed between AFB1 level in blood serum and infected with chronic kidney disease (CKD). [1] some studies found relation Ship between dose of AFB1 and grain. of infants in Gambia, Benin and Togo.[7]

Table (7) Estimation of correlation coefficient (r)

Type of Correlation	Coefficient (r)	P. Value
Gender ×blood toxin (AFB 1)	0.974	0.324
Health Status X blood toxin (AFB 1)	0.703	0.402

The results illustrated that creatinin levels in blood (M, D-2, Tx) group was 1.88 mg/dL while the levels of creatinine in blood of (F,D-2,Tx), (F,D-2,NT) and (F,H, NT) groups were (0.59,0.57, 60) mg/dL respectively with significant difference between them. on the other hand the croatinine levels in blood of (M , H , Tx), (M, H, NT) and (F, H_2 Tx) group were (0.73,0.71,0.71) mg/dL respective with out significant difference (Table _8). several studies agreement with result of this study .study found that createriuine level in blood females patients with nephropathy and contaminated with ochraloxin A increases to 3.73 mg/dL while the level of creatinine in blood healthy females was 0.57 mg/dL on the other hand the levels of creatinine in blood serum of males patients reached 4.64 mg/dL. [4]Also of the other study illustrated that creatinine levels in blood serum of Patients With Chronic kidney disease and contamination with AFB1 get 2.29 mg/dl [1]

Study have [14]demonstrated that AFB1 Caused injury in kidney tissue and inflammatory cell infiltration , hemorrhage damage and necrosis

Table (7) Creatinine levels in blood serum of groups Study.				
No. of group	groups	Statistical Notation	Mean mg/dL	SD±
1	M,D-2,Tx	b	1.88	0.13
2	M,D-2,NT	a	0.66	0.06
3	M,H,Tx	ab	0.73	0.28
4	M,H,NT	a	0.71	0.17
5	F,D-2,Tx	a	0.59	0.18
6	5D-2,NT	a	0.57	0.04
7	F,H,Tx	ab	0.71	0.15
8	F,H,NT	a	0.60	0.14

Number with difference letters mean that there are significant difference between them at $P < 0.05$.
According statistical analysis of uric acid levels shown there is significant difference among the means of group study. Females patients with diabetic disease and blood its contamination with AFB1 the level uric acid was 8-3 Mg/dl with significant difference of same group As 4,7 and 8 group. Levels of urea in blood were (5.78 , 5.82 , 5.76) mg/dl respectively . while there is not significant difference in others groups (table _8)

Table (8) uric acid levels in blood serum of groups study				
No. of groups	groups	Statistical notation	Mean mg/dl	SD ±
	M,D-2,TX	b	8.3	3.44
	M,D-2,NT	ab	7.73	0.66
	M,H,TX	ab	7.76	2.62
	M,H,NT	a	5.87	1.37
	F,D-2,TX	ab	7.77	0.85
	F,D-2,NT	ab	6.68	0.54
	F,H,TX	a	5.82	0.33
	F,H,NT	a	5.76	1.39

Numbers with different letters mean. That there are significant differences between them at $p<0.05$
The results that shown in table (9) illustrated the levels of urea in blood (M, D-2, TX) group and (M, D-2, NT) was 42 mg/dL and 40 mg/dL respectively. The levels of urea in blood of first group was significant difference from levels of other groups. Also levels of urea in blood of group one and two was abnormal. On other hand the levels of urea in blood of groups (2,3,4,5,6) were not significant diferace between them. The levels in blood groups 4, 7, 8 were (34, 32.62, 30.25) mg/dL respectively without significant difference between them (Table 7_4)
The result of this study agreement with some studies that found urea and creatinine levels increased because the correlate with variations in glomerular rate. Blood urea levels become high levels when renal function decline to about 25 – 50 % the researchers found that increase level of urea indicating serious kidney damage. Also blood levels of urea considerably higher, whereas protein level are lower than the Control group indicating that kidney funcion is impaired[5].

Table (9) urea levels in blood serum of group study.				
No. of group	groups	Statistical Notation	Mean mg/dL	SD ±
1	M,D -2,TX	a	42	17.82
2	M, D -2, NT	ab	40	10.41
3	M,H ,T x	b	38	11.31
4	M, H, NT	bc	34	9.06
5	F ,D -2, Tx	b	35	13.97
6	F,D -2, NT	b	36	10.41
7	F ,H ,Tx	c	32.62	5.62
8	F , H , NT	c	30.25	3.62

Numbers with different letters mean that there are significant difference between them at P < 0.05.

The results of this test showed that levels of ALP in blood seven of [M,H, Tx), [(M, D_2, NT), (F,D-2, NT), (F,D-2 Tx)] groups were (147.1,145. 28,145.8, 138.3) U/L respectively with significant difference from other groups. While the levels of ALP in blood serum of (M, H, NT) group and (F, H, NT) group were (67.9 u/L) and (57.4 u/L) respectively

As well as the groups of number (1) and (7) was normal (Table _9).

This results [14] agree with result which found that mycotoxin increased (ALP) levels in blood serum of patients will nephropathy and carrier toxin with significant difference of ALP level in blood healthy groups.

The results/ found that mean ALP level in blood serum of patient with CKD and Contamination with AFB1 was 110.18 U/L While the level in blood healthy group 78 U/L.[1]

The results showed that AST level in male patients with diabetic type-2 and the blood serum not contamination with AFB1 (group/2) and females patients with diabetic type-2 and there is no toxin (AFB1) in blood (group/6) was 53.38 U/L and 49. 82U/L respectively. while the levels of AST in blood Serum of(males healthy) without AFB1 group and the levels in blood serum of (females, healthy, without AFB1 toxin) decreased to (19.40) U/L and (21.50) U/L receptively with significant difference between them. Also the levels in blood serum of other groups were between (37. 20to 95. 44) U/L (Table _10)

Table (10) ALP levels in blood serum of groups study.

No. of group	groups	Statistical Notation	Meanu U/L	SD±
1	M,D-2,Tx	b	115.4	31. 45
2	M,D-2,NT	c	145.8	29. 05
3	M,H,Tx	c	147.1	33. 27
4	M,H,NT	a	67.9	27.7
5	F,D-2,Tx	c	138.3	31. 24
6	F, D-2 ,NT	c	145.28	36. 48
7	F, H , Tx	b	114.8	38. 11
8	F ,H, NT	a	57.4	27.8

Numbers with different letters mean that there are significant difference between them at P < 0.05.

Table (11). AST levels in blood serum of groups Study

No-ol group	group	Statistical Notation	Mean U/L	SD±
1	M,D-2,Tx	ab	42.14	30. 48
2	M,D-2,NT	b	53. 38	29. 86
3	M,H,Tx	ab	37. 20	31. 85
4	M,H,NT	a	19. 40	5. 38
5	F,D-2,Tx	ab	45. 44	26. 94
6	F, D-2 ,NT	b	49. 82	34. 22
7	F, H , Tx	ab	41. 63	31.34
8	F ,H, NT	a	21. 50	2. 34

Numbers with different letters mean that there are significant difference between them at P < 0.05

Table(12) ALT levels in blood serum of groups study.				
No-ol group	group	Statistical Notation	Mean U/L	SD±
1	M,D-2,Tx	a	18. 159	3. 21
2	M,D-2,NT	b	15. 08	19
3	M,H,Tx	ab	12	7. 18
4	M,H,NT	ab	38	4. 48
5	F,D-2,Tx	ab	13. 63	5. 45
6	F, D-2 ,NT	b	14. 83	6. 73
7	F, H , Tx	ab	88	5. 28
8	F ,H, NT	ab	9. 5	2. 22

Numbers with different letters mean that there are significant difference between them at $P < 0.05$
Regarding to statistical analysis of ALT shows there is significant differences among of study groups.
ALT levels in the blood serum of (M. D_2,TX) group reached to (18.195) U/L while the levels of ALT in blood for (M, H, TX), (M, H, NT), (F, D_2,TX) and (F, H, TX) groups were (12, 10.38, 13.63, 12.88) U/L respectively. (Table _11) without significant differences between them at P(0.023).
There are many results approached of this study. Some researchers found that the range of ALT level in blood serum patients with CKD diseases and exposure to AFB1 was (5.51-23.84) U/L compare with level in blood serum of healthy persons (12.07-21.84)U/L. The result of local study demonstrated the ALT level in blood serum of Patients with CKD and exposure to OTA toxin was 9.98 U/L Compare with level of ALT in blood serum of healthy (7.86 U/L) with no Significant difference between them [1]
The ALT enzyme is typically found in liver cells/ it's presence in high level in Plasma implies tissue damaged or organ failure

6. Conclusion

The presence of aflatoxin B1 in blood serum of human at any concentration is a dangerous indicator of human health, because this toxin is accumulative and has many vital goals in the human body. The relationship between aflatoxin B1 and diabetes disease was synergism to increase levels of AST and ALT. According to available sources, this study is the first of it's Kind locally and globally.

7. References

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