3. RESULTS AND DISCUSSION

Many fungi that grow and contaminate stored foodstuffs are known to produce substances as secondary metabolites which exhibit harmful effects on various body tissues when consumed by animals or man. Aflatoxin B_1 is the most abundant secondary fungal metabolite produced by certain strains of *Aspergillus flavus, A. parasiticus*. It is the most potent hepatocarcinogen so far recognized, and is suspected of being a primary cause of human cancer in certain areas of the body **(Wogan and Newbrene, 1967)** and is involved in the etiology of fiver disease.

Generally, the rat is considered to be the most sensitive mammal to aflatoxin carcinogenesis.

Due to the essential role of the liver in the metabolism and detoxification of a wide range of toxic materials and that of the kidney in eliminating the waste products from the body, liver and kidneys were selected as target organs.

The choice of these mycotoxins, was due to their common occurrence in food and foodstuffs. Due to the fact that chronic ingestion of small amounts of toxins has more significance than acute exposure **(Doerr et al., 1983)**.

3.1 Fungi isolated from seeds sample of peanut

Table (4) showed that the fungi isolated from seeds are *Aspergillus flavus, Aspergillus niger, Fusarium solani, Pencillium funiculosum* and *Rhizoctonia solani*.

The results indicated that *Fusarium solani* are the dominant fungi (25.64 %) followed by *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp.* These results agree with **Bullerman (1979)**.

3.2 Fungi isolated from grains sample of corn

Table (5) showed that the fungi isolates from corn are *Aspergillus flavus, A. parasiticus, A. niger, Rhizopus oryzae, Mucor racemosus, Penicillium corylophilum* and *Alternaria allternata*.

Aspergillus niger is the dominant fungus followed by *Aspergillus flavus*. These results agree with **Bokhary and Naguib (1983)**.

3.3 Screening of aflatoxin production by various isolates of fungi isolated from peanut and corn grown in liquid medium.

Table (6), showed that a total of (13) isolates of *Aspergillus flavus* were screened for aflatoxin production. It was cleared that (7) isolates produced aflatoxin B_1 (No. 100, 101, 102, 103, 104, 105 and 106), three isolates produced aflatoxin B_2 (No. 107, 108 and 109) and three isolates only produced more than one aflatoxin (classified as follow: one isolate No. 110 produced $AFTB_1$ & $AFTG_1$, one isolate No. 111 produced $AFTB_2$ & $AFTG_2$ and one isolate No. 112 produced $AFTB_1$ & $AFTB_2$).

The result obtained from the same table showed that (17) isolates of *Aspergillus niger* were screened for aflatoxin production, all the isolates failed to produce aflatoxin in the used yeast extract sucrose medium. And the same table showed that seven isolates of *Penicilium* were screened for aflatoxin production, all the *Pencillium* species failed to produce aflatoxin. In the same table showed that three isolates of *A. parasiticus* were screened for aflatoxin production, two isolates produce aflatoxin B_1 & B_2 and the other isolate produce aflatoxin B_1 & G_1, in the same table, two isolates of *Alternaria allternata* failed to produce aflatoxin. Ten isolates of *Fusarium spp*., all the isolates failed to produce aflatoxin. All the other fungi present in the same table failed to produce aflatoxin in the used YES medium.

3.4. (Change in blood) Biochemical observations

The biochemical events have been reviewed by **Busby and Wogan (1984)**. Covalent binding of aflatoxin to nucleic acid occurs within minutes of treatment an animal with AFB_1 and results in a precipitatous decrease in both DNA and RNA synthesis rates in the liver. The degree of inhibition is even more pronounced in a liver in which parenchymal cells have been stimulated to divide as a result of partial hepatectomy **(Rogers et al., 1971; Neal and Cabral, 1980)**. Nucleic acid synthesis only begins to recover after 1-2 days. Protein synthesis is inhibited by AFB_1, but not as rapidly or as extensively as that of nucleic acids. Within 1 hr or so after AFB_1 exposure, protein synthesis declines. Polysomal disaggregation parallels this inhibition and is likely to represent the mode of inhibition of protein synthesis. Reaggregation of ribosomes and return of protein synthesis to normal occurs within 2-5 days of acute aflatoxin treatment. Lipid accumulation in response to aflatoxin exposure occurs in surviving parenchymal cells and returns to normal even more slowly than do nucleic acid and protein synthesis. This latter effect is probably secondary to the inhibition of synthesis of proteins for transport of lipids from the hepatcyte. Aflatoxin increased the glucose level and decreased the cholesterol level in blood significantly. Levels of blood triglyceride, total protein and albumin were not affected by aflatoxin, vitamin E or Se.

Activities of blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased by aflatoxin. The vitamin E supplementation decreased the AST activity significantly. The levels of total cholesterol and free cholesterol in the liver were significantly lower in the rats receiving aflatoxin. A significant increase in the activities of ALP, ALT and AST was recorded in AFT treated group compared with control group. With respect to vitamins

supplementation groups, it is observed that ALP activity was decreased significantly in β-carotene, vitamin C and vitamin E supplemented groups. ALT activity was markedly dropped in all vitamins supplemented rats. Whereas, AST activity was decreased significantly in β-carotene group. Meanwhile, BUN and creatinine were decreased in vitamin A supplmeneted rats. Also BUN was significantly decreased with β-carotene treatment compared with AFT treated group (Table 15-19).

3.4.1. Effect on activities of plasma enzymes (ALP, ALT and AST):

The activities of plasma ALP, ALT and AST enzymes of rats given AFT contaminated diet were significantly increased. This may be due to necrosis of hepatic cells and release of these enzymes into circulation **(Lynch et al., 1971)**. The hepatic changes could be attributed to the acute toxic effect of AFT which is converted in the liver to several toxic metabolites including epoxide. This metabolite is thought to be the active toxic form or an ultrimate carcinogen **(Brucato et al., 1986 and Netke et al., 1997)** which covalently bind to DNA. This adduct formation is regard as the primary and critical events in the $AFTB_1$ carcinogenesis.

3.5. Biochemical analysis of blood of rats which injected with aflatoxin B_1:

The results indicated that, the liver of the treated rats underwent malfunction during the treatment period as reflected by the total bilirubin increase and or the periportal necrosis which elevated the serum bilirubin concentration **(Clifford and Rees, 1967)**.

3.5.1. Change in total protein:

Results of the biochemical analysis pattern of rats injected with aflatoxin B_1 (AFB_1) at a dose level of 0.5 mg/kg B.W., revealed that there was a significant decrease ($P < 0.05$) in total protein which may be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis. **Osuna and Eddes (1982)** suggested that such effect may be attributed to the metabolism of aflatoxin in the liver, where it interferes with protein synthesis and RNA production, resulting in decreasing albumin and β- and γ-globulins. In addition, the reduction in serum proteins resulted from aflatoxin administration is due to the damage caused by the toxin in the liver (Table 14).

3.5.2. Change in total cholesterol:

Regarding the changes in serum total cholesterol the results showed significant decrease in total cholesterol level, these results are in agreement with that of **Lanza et al., (1980**) (Table 13).

3.5.3. Change in bilirubin and urea:

The increase in the concentration of bilirubin in the rats injected with AFB_1 were significant high when compared to that of the mean control value. These results are in compatible with **Derell et al., 1982**. The results show that, the urea level was increase, and these increase of urea level may be ascribed to the effect of the toxin on liver and kidney functions, as urea is the end product of protein catabolism **(Coles, 1986)** (Tables 15 & 16).

3.5.4. Change in creatinine and plasma enzymes (ALT & AST):

The high level of the urea in blood serum resulted from either the increase in breakdown to tissue protein of impairment of urea excretion

(Bush, 1991). And the results show the significant increase in creatinine value in the serum of aflatoxin B_1 treated group. Concerning the activity of serum enzyme alanine aminotransferase (ALT) in AFB_1 treated groups. The activities of ALT in all treated groups were significantly increased from those control group. The mean values of serum enzyme aspartate aminotransferase (AST) in AFB_1 administered groups were significantly increased from those of the control groups. The elevation of ALT and AST levels may be due to the increase permeability of the liver cell leading to the release of the transferases into the blood stream. This effect is a result of the hepatocellular injury of liver cell membrane induced by the toxins **(Duncan and Prasse, 1977)**. The observed increase in the measured enzyme activities suggests that exposure to AFB_1 is connected with impairment of the liver and kidney functions (Tables 17-19).

Table (4): Genera and species of fungi isolated from seeds samples of peanut (*Arachis hypogaea L.*).

Fungal species	Number of isolates	Percent of frequency (incidence)
Aspergillus flavus (Link: Fr)	7	17.948
A. niger (Van Tieghem)	9	23.076
Fusarium solani (Mart) Sacc.	10	25.641
Penicillium funiculosum (Thom)	5	12.820
Rhizoctonia solani (Kühn)	8	20.512
Total count	39	100

The results agree with ***Bullerman (1979)*** isolated *Aspergillus sp*. (*A. flavus, A. ochraceus, A. versicolor, A. parasiticus*). *Penicillium sp. (P. cyclopium, P. expansum, P. citrinum, P. viridicatum, P. urticae, P. islandicum). Alternaria, Cladoporium, Fusarium* and *Rhizopus* from peanut, dry beans and soybeans.

Table (5): Show genera of fungi isolated from grains sample of corn (*Zea mays*).

Fungal species	No. of isolates	Percent of frequency (incidence)
Aspergillus flavus (Link: Fr)	6	25
A. parasiticus Spear	3	12.5
A. niger (Van Tieghem)	8	33.333
Rhizopus oryzae (Went & Prinsen Geerlings)	2	8.333
Mucor racemosus (Fresenius)	1	4.166
Penicillium Corylophilum (Dierckx)	2	8.333
Alternaria allternata (Fries) Keissler	2	8.333
Total count	24	100

The results agree with those obtained by ***Bokhary and Naguib (1983)*** which isolated species of *Aspergillus flavus, Alternaria* and *Fusairum* from corn and other sources. And ***Shank et al. (1972)*** who found that *Aspergillus flavus* was the predominating fungus while *Rhizopus*, *Pencillium* and *Fusarium* were only present sometimes on beans and corn.

Table (7): Effect of vitamins supplementation on body and some organs weight of male rats fed on aflatoxins contaminated diet (Mean ± S.E).

Treatment of experimental animals	Relative weight percentage $(\overline{X} \pm S.E)$				
	Body weight	Liver	Kidney	Heart	Spleen
Group 1 (Control) AFT-ve	265.2 ± 1.772	6.76 ± 0.1406	1.26 ± 0.0442	0.662 ± 0.0058	0.468 ± 0.0037
Group 2 AFT +ve	231.8 ± 1.462	5.76 ± 0.0667	1.83 ± 0.0267	0.51 ± 0.0044	0.322 ± 0.0066
Group 3 AFT + Vit. A	236.4 ± 1.208	6.47 ± 0.0185	0.84 ± 0.0118	0.548 ± 0.0051	0.366 ± 0.0050
Group 4 AFT+ β-Carotene	246.4 ± 1.363	6.59 ± 0.0236	1.12 ± 0.0102	0.580 ± 0.0070	0.398 ± 0.0066
Group 5 AFTP + Vit.C	216.4 ± 1.208	6.09 ± 0.0396	0.91 ± 0.0097	0.522 ± 0.00374	0.342 ± 0.00374
Group 6 AFT + Vit.E	239.2 ± 1.019	6.63 ± 0.0382	0.97 ± 0.0058	0.628 ± 0.00374	0.44 ± 0.0070

- The results showed decrease in liver and body weight, while kidney weight were increased in aflatoxin treated group as compared with control group.
- **While in vitamins supplemented groups**
  - A non significant increase in body weight was recorded in groups supplemented with vitamins A, β-carotene and vitamin E. And there are increase in liver weight after supplementation with vitamin E was recorded, and the kidney weight of rats treated with vitamin A, β-carotene and vitamin C was decrease.
  - A decrease in liver weight due to aflatoxicosis was similar with ***Espada et al., (1992)*** in broilers. This change in the weight may

be due to damage of hepatocytes or because the liver is the target organ for aflatoxin metabolism.

- Supplementation with vitamins (especially vitamin C and E) leads to increased body and liver weight. The protective activity of ascorbic acid was due to induction of enzyme $AFTB_1$-epoxide hydrolase or aldehyde or aldehyde reductase or both ***(Netke et al., 1997)***.
- Vitamin E protect cells from damage by conjugating $AFTB_1$-epioxide shortly after their formation and thus preventing these reactive metabolites from binding to essential cellular protein or nucleic acids with subsequent reduction in AFT metabolism ***(Brucato et al., 1986 and Ibeh and Saxena, 1998)***.
- A marked drop in the body weight of rats treated with AFT was observed and these results was similar with ***(Brucato et al., 1986 and Hendrickse, 1991)***, this drop may be due to decreased in the nitrogen absorbability in the diet as showed by ***Reddy et al., (1991)*** or loss of appetite and diarrhea ***(Cook et al., 1989)*** and the effect on liver capacity for protein synthesis ***(Wogan, 1975)***.

Table (8): Effect of vitamins supplementation on the activity of plasma alkaline phosphatase (ALP) of male rats fed on diet contaminated with aflatoxins for one month (Mean ±S.E).

Treatment (groups)	Alkaline phosphatase (ALP) (u/ml)		
	Feeding period (days) ($\overline{X}$ ± S.E.)		
	10* day	20* day	30* day
Group (1) AFT (-ve)	118.5 ± 0.741	119 ± 1.095	119.4 ± 1.077
Group (2) AFT (+ve)	160.2 ± 0.663	163.8 ± 0.582	168.2 ± 1.280
Group (3) AFT + vit (A)	140.6 ± 0.871	144.8 ± 0.663	150.2 ± 1.240
Group (4) AFT + β-carotene	119.4 ± 0.87	123. ± 1.067	128 ± 2.097
Group (5) AFT + vit. (C)	126.2 ± 0.799	129.8 ± 0.663	136 ± 1.414
Group (6) AFT + vit. (E)	118.6 ± 0.8716	120.8 ± 0.373	122.6 ± 0.509

Value at zero time of ALP = 118.5 u/mL

* = after feeding

- The results showed that there are increase in the activity contaminated diets compared with control group.
- In vitamins supplemented groups, the results show that ALP activity was decreased in β-carotene, vitamin C and vitamin E supplemented groups of contaminated diets without vitamins supplementation .

- The increase in the activity of ALP may be due to necrosis of hepatic cells and the release of these enzymes into the circulation **(Lynch et al., 1971).** Such hepatic changes could be attributed to the acute toxic effect of aflatoxin which is converted in the liver to several toxic metabolites including epoxide. This metabolite is thought to be the active toxic form or an ultimate carcinogen **(Brucato et al., 1986 and Netke et al., 1997)** which convalently bind to DNA. This adduct formation is regard as the primary and critical events in the AFT B_1 carcinogenesis.

In Vitamins supplementation groups:

Especially vitamin C and vitamin E leads to reduced activity of ALP. The protective activity of ascorbic acid was due to induction of enzyme $AFTB_1$-epioxide hydrolase or aldehyde reductase or both **(Netke et al., 1997)**.

Vitamin E protect cells from damage by conjugating $AFTB_1$-epioxide shortly after their formation thus preventing these reactive metabolites from binding to essential cellular protein or nucleic acids with subsequent reduction in AT metabolism **(Brucato et al., 1986 and Ibeh and Saxena, 1998)**.

Table (9): Effect of vitamins supplementation on the activity of plasma alanine amino-transferase (ALT) of male rats fed on diet contaminated with aflatoxins for one month (Mean ±S.E).

Treatment (groups)	Alanine amino-transferase (ALT)		
	Feeding period (days) ($\bar{X}$ ± S.E.)		
	10* day	20* day	30* day
Group (1) AFT (-ve)	36.3 ± 0.538	36.8 ± 0.583	37.4 ± 0.187
Group (2) AFT (+ve)	45.8 ± 0.860	53.8 ± 0734	64 ± 0.707
Group (3) AFT + vit (A)	37.04 ± 0.244	38.8 ± 0.373	41.8 ± 0.799
Group (4) AFT + β-carotene	38.8 ± 0.583	42 ± 0.948	46.6 ± 0.509
Group (5) AFT + vit. (C)	39.6 ± 0.678	43.2 ± 0.916	48 ± 0.894
Group (6) AFT + vit. (E)	40.6 ± 0.871	47.2 ± 0.860	52.8 ± 0.853

Value at zero time of ALT = 36.3 u/mL

S.E. = Standard error

* = after feeding

The activity of plasma ALT increase in AFT treated group compared with control group.

In vitamins supplementation groups:

The results show that ALT was decrease in all vitamins supplemented rats compared with

The increase in the activity of plasma ALT enzyme of rats given AFT-contaminated diet may be due to necrosis of hepatic cells and the release of these enzymes into the circulation ***(Lynch et al., 1971)***.

Table (10): Effect of vitamins supplementation on the activity of plasma aspartate aminotransferase (AST) of male rats fed on diet contaminated with aflatoxins for one month (Mean ±S.E).

Treatment (groups)	Aspartate aminotransferase (AST) (u/ml)		
	Feeding period (days) ($\overline{X}$ ± S.E.)		
	10* day	20* day	30* day
Group (1) AFT (-ve)	159.8 ± 0.374	160.8 ± 0.374	161.8 ± 0.583
Group (2) AFT (+ve)	173.6 ± 0.509	181.6 ± 0.927	190.6 ± 1.076
Group (3) AFT + vit (A)	164.4 ± 0.509	168.2 ± 0.800	174.4 ± 0.678
Group (4) AFT + β-carotene	162.8 ± 0.372	165.0 ± 0.447	168 ± 0.707
Group (5) AFT + vit. (C)	164.8 ± 0.800	174.2 ± 0734	180.6 ± 0.878
Group (6) AFT + vit. (E)	163.0 ± 0.447	165.4 ± 0.509	170.6 ± 1.076

S.E. = Standard error

* = After feeding

value at zero time of AST = 159.8 u/mL

The activity of plasma AST in male ratio fed on AFT contaminated diets was increase in AFT treated group compared with control group.

AST activity was decreased in β-carotene group.

The activity of plasma AST enzyme of rats given AFT contaminated diet were increased. This ma be due to necrosis of hepatic cells and release of these enzymes into the circulation ***(Lynch et al., 1971)***.

Table (11): Effect of vitamins supplementation on the activity of blood urea nitrogen (BUN) of male rats fed on diet contaminated with aflatoxins for one month (Mean ±S.E).

Treatment (groups)	Blood urea nitrogen (BUN) (mg/dI)		
	Feeding period (days) ($\overline{X}$ ± S.E.)		
	10* day	20* day	30* day
Group (1) AFT (-ve)	12.7 ± 0.435	13.04 ± 0.1631	13.4 ± 0.1449
Group (2) AFT (+ve)	15.16 ± 0.094	15.78 ± 0.2455	16.98 ± 0.367
Group (3) AFT + vit (A)	14.2 ± 0.094	14.6 ± 0.114	15.2 ± 0.2429
Group (4) AFT + β-carotene	14.16 ± 0.0509	14.46 ± 0.132	15.3 ± 0.200
Group (5) AFT + vit. (C)	14.46 ± 0.1886	15.32 ± 0.1067	15.76 ± 0.102
Group (6) AFT + vit. (E)	14.5 ± 0.1774	15.8 ± 0.080	16.8 ± 0.1224

S.E. = Standard error

* = After feeding

value at zero time of BUN = 12.7 mg/dI

Concentrations of total BUN in male rats fed on AFT contaminated diets was increased in aflatoxin treated group compared with control group.

BUN were decreased in vitamin A supplemented rats and also BUN was decreased with β-carotene treatment compared with AFT treated group affections which appear in kidney **(Mobarak et al., 1996)** and tubular due to direct nephrotoxic effect of AFT.

Table (12): Effect of vitamins supplementation on the creatinine level of male rats fed on diet contaminated with aflatoxins for one month (Mean ±S.E).

Treatment (groups)	**Creatinine (mg/dI)**		
	Feeding period (days) ($\overline{X}$ ± S.E.)		
	10* day	20* day	30* day
Group (1) AFT (-ve)	0.88 ± 0.0070	0.89 ± 0.0044	0.90 ± 0.0054
Group (2) AFT (+ve)	0.988 ± 0.0058	1.016 ± 0.0050	1.04 ± 0.0070
Group (3) AFT + vit (A)	0.922 ± 0.0073	0.944 ± 0.0050	0.976 ± 0.00509
Group (4) AFT + β-carotene	0.922 ± 0.0066	0.928 ± 0.00374	0.95 ± 0.0070
Group (5) AFT + vit. (C)	0.962 ± 0.0037	0.986 ± 0.0050	1.016 ± 0.0050
Group (6) AFT + vit. (E)	0.972 ± 0.00374	0.992 ± 0.0038	1.06 ± 0.00244

S.E. = Standard error

* = after feeding

value at zero time of creatinine = 0.89 mg/dI

The results show that creatinine was decreased in vitamin A supplemented rats.

And also the results show that creatinine was increase in aflatoxin treated group compared with control group. This effect may be attributed to glomular and tubular affections which appear in kidney. Such alterations were in parallel line with observations of **Rati et al. (1991)** and **Mobarak et al., (1996)** and could be due to direct nephrotoxic effect of AFT.

Table (13): Effect of aflatoxin B_1 on plasma total cholesterol of albino male rats injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	Plasma total cholesterol $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	10* day	20* day	30* day
Group 1 Control	84.8 ± 0.374	85.2 ± 0.374	85.6 ± 0.509
Group 2 0.2 ml saline solution 9%	84.5 ± 0.763^{A}	81.5 ± 0.428^{A}	81.16 ± 0.401^{A}
Group 3 0.2 ml of 7% Tween-80	80.66 ± 0.333^{B}	77.66 ± 0.666^{B}	74.83 ± 0.792^{B}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	70.00 ± 0.577^{C}	65.83 ± 0.600^{C}	58 ± 0.856^{C}
LSD	0.198	0.22	0.341

Zero time value of cholesterol = 86 mg /dI

* = After feeding

B.W. = Body weight

The results showed significant decrease in total cholesterol level these results are in agreement with that of **Lanza et al., (1980)**.

Table (14): Effect of aflatoxin B_1 on total protein of albino male rats injected aflatoxin B_1 interperitoneal (IP) twice a week for one months.

Treatment	Total Protein $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	*10 day	*20 day	*30 day
Group 1 Control	6.26 ± 0.0509	6.28 ± 0.037	6.28 ± 0.037
Group 2 0.2 ml saline solution 9%	6.25 ± 0.0427^{A}	6.23 ± 0.033^{A}	6.11 ± 0.308^{A}
Group 3 0.2 ml of 7% Tween-80	5.75 ± 0.076^{A}	5.36 ± 0.049^{A}	4.26 ± 0.0843^{B}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	4.18 ± 0.060^{B}	3.75 ± 0.076^{B}	3.03 ± 0.0385^{C}
LSD	0.54	0.12	0.11

Zero time value of protein = 6.50 mg /dI

* = After feeding

B.W. = Body weight

AFB_1 (0.5 mg/kg) induced a significant decrease ($p < 0.05$) in total protein which may be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis. **Osuna and Eddes, 1982**, suggested that such effect may be attributed to the metabolism of aflatoxin in the liver, where it interferes with protein synthesis and RNA production, resulting in decreasing albumin and β and γ globulins. In addition, the reduction in serum proteins resulted from aflatoxin administration is due to the damage caused by the toxin in the liver.

Table (15): Effect of aflatoxin B_1 on bilirubin of albino male rats injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	Bilirubin $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	*10 day	*20 day	*30 day
Group 1 Control	0.46 ± 0.004	0.462 ± 0.0058	0.464 ± 0.0060
Group 2 0.2 ml saline solution 9%	0.46 ± 0.0036^{A}	0.47 ± 0.0036^{A}	0.48 ± 0.0048^{A}
Group 3 0.2 ml of 7% Tween-80	0.47 ± 0.006^{A}	0.54 ± 0.013^{B}	0.62 ± 0.018^{B}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	0.60 ± 0.0106^{B}	0.87 ± 0.052^{C}	1.66 ± 0.096^{C}
LSD	0.089	0.215	0.231

Zero time value of bilirubin = 0.46 mg /dI

* = After feeding

B.W. = Body weight

The results show that, the increase in the concentration of bilirubin in the rats injected with AFB_1, these results are in agree with **Derell et al., (1982)**. The results indicated that the liver of treated rats underwent malfunction during the treatment period as reflected by the total bilirbuin increase.

Table (16): Effect of aflatoxin B_1 on urea of albino male rats injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	Urea $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	*10 day	*20 day	*30 day
Group 1 Control	15.58 ± 0.0374	15.6 ± 0.054	15.6 ± 0.0316
Group 2 0.2 ml saline solution 9%	15.61 ± 0.030^{A}	15.65 ± 0.022^{A}	15.7 ± 0.036^{A}
Group 3 0.2 ml of 7% Tween-80	15.75 ± 0.042^{A}	15.96 ± 0.055^{A}	16.36 ± 0.049^{A}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	17.11 ± 0.345^{B}	19.23 ± 0.229^{B}	21.28 ± 0.319^{B}
LSD	0.179	0.0982	0.110

Zero time value of urea = 15.6 mg /dI

* = After feeding

B.W. = Body weight

At the end of the experiment period a significant increase in serum urea was detected in AFB_1 (0.5 µg/kg of BW) administrated groups than that of the control one. The increase of urea level may be due to the effect of the toxin on liver and kidney functions, as urea is the end product of protein catabolism **(Coles, 1986)**. The high level of urea in blood serum resulted from either the increase in breakdown of tissue protein of impairment of urea excretion **(Bush, 1991)**.

Table (17): Effect of aflatoxin B_1 on creatinine of albino male rats male injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	Creatinine $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	* 10 day	*20 day	*30 day
Group 1 Control	0.81 ± 0.0070	0.814 ± 0.0207	0.816 ± 0.008
Group 2 0.2 ml saline solution 9%	0.82 ± 0.0079	0.85 ± 0.0057	0.86 ± 0.0048
Group 3 0.2 ml of 7% Tween-80	0.84 ± 0.0079	0.86 ± 0.0036	0.88 ± 0.0057
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	0.89 ± 0.0114	0.99 ± 0.018	2.02 ± 0.1302
LSD	0.0792	0.39	0.31

Zero time value of creatinine = 0.81 mg/dI

* = After feeding

B.W. = Body weight

The results show the significant increase in creatinine value in the serum of AFB_1 treated groups.

Table (18): Effect of aflatoxin B_1 on (AST) asparate aminotransferase of albino male rats injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	(AST) $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	* 10 day	*20 day	*30 day
Group 1 Control	181 ± 0.447	181.2 ± 0.583	181.2 ± 0.374
Group 2 0.2 ml saline solution 9%	182 ± 0.577^{A}	185 ± 0.516^{A}	186.5 ± 0.428^{A}
Group 3 0.2 ml of 7% Tween-80	186 ± 0.683^{B}	191.5 ± 0.763^{B}	194 ± 0.577^{B}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	191 ± 0.966^{C}	195.5 ± 0.763^{C}	199.5 ± 1.176^{C}
LSD	0.0982	0.0159	0.321

Zero time value of AST = 181 u/I

* = After feeding

B.W. = Body weight

The activity of serum enzyme asparatate aminotransferase (AST) in AFB_1 groups were significantly increased from those of the control groups. The elevation of ALT and AST levels may be due to the increase permeability of the liver cell leading to the release of transferases into the blood stream. This effect is a result of the hepatocellular injury of liver cell membrane induced by the toxins **(Duncan and Prasse, 1977)**. The observed increase in the measured enzyme activities suggested that exposure to AFB_1 is connected with impairment of the liver and kidney functions.

Table (19): Effect of aflatoxin B_1 on (ALT) alanine aminotransferase of albino male rats male injected aflatoxin B_1 interperitoneal (IP) twice a week for one month.

Treatment	ALT $(\overline{X} \pm S.E)$		
	Feeding period (days)		
	*10 day	*20 day	*30 day
Group 1 Control	48 ± 0.707	48.2 ± 0.734	48.2 ± 0.663
Group 2 0.2 ml saline solution 9%	48.5 ± 0.428^{A}	50.5 ± 0.428^{A}	51.83 ± 0.477^{A}
Group 3 0.2 ml of 7% Tween-80	52.2 ± 0.946^{B}	56.83 ± 0.602^{B}	62.5 ± 0.763^{B}
Group 4 0.5µg /kg B.W. AFT B_1 dissolved in 7% Tween-80	56.83 ± 1.077^{C}	64.5 ± 0.763^{C}	70 ± 1.064^{C}
LSD	0.115	0.0382	0.0760

Zero time value of ALT = 48 u/I

* = After feeding

B.W.= Body weight

The activity of serum enzyme alanine aminotransferase (ALT) in AFB_1 groups were significantly increased from those control group. The elevation of the ALT level may be due to the increase permeability of the liver cell leading to the release of the transferases into the blood stream. This effect is a result of the hepatocellular injury of the liver cell membrane induced by the toxins **(Duncan and Prasse, 1977)**.

3.6. Histopathological changes:

3.6.1. Rats liver:

Toxin injected in a dose of 0.5 mg aflatoxin B_1/kg body weight for one month in 150-200 gm adult male rats produce cloudy degeneration of hepatocytes, areas of necrosis, congested dilated central veins, warked sinusoidal dilation and kupffer cell hyperplasia. This is an agreement with **Clark et al. (1982)** who explained increased cytoplasmic easihophilia and loss of cytoplasmic granularity of hepatocytes. The portal tracts showed telangiectatic changes in the blood vessels and proliferation of bile ducts.

Yin et al., (1980) stated that in the rat the hepatotoxic effects of aflatoxin can be correlated with the circulatory pattern of the hepatic lobule as evidenced by periportal necrosis and biliary hyperplasia. Our results revealed mild thickening of he portal tracts by mononuclear inflammatory cells. However, **El-Zahan et al., (1996)** recorded periportal fibrosis with degeneration and ulceration of bile duct epithelial cell lining and proliferation of bile ducts.

The microcopical examination of rats liver treated with aflatoxin only (AFT +ve) revealed vacuolar degeneration of hepatic cells and diffused fatty change scattered allover the hepatic parenchyma. In some cases perilobular fibrosis associated with hepatic cell necrosis and infiltration with mononuclear cell mainly lymphocytes and macrophages were seen. The affected liver showed obvious increase general in size of some hepatocytes and their nuclei (megalocytosis) associated with presence of intra nuclear inclusions. Dilatation and congestion of hepatic blood vessels in addition to moderate hyperplasia of epithelial lining of bile duct were noticed. Activation of Kupffer cells was observed.

The pathological picture of rat liver in group (vit. A and β-carotene) was focal areas of vacuolar degeneration and fatty change. In addition to these lesions, the centrolobular hepatocytes were necrosed and replaced by mixture of inflammatory cells mainly lymphocytes and fibroblasts. Necrosis and desquomationof the biliary ductal epithelium and excessive aggregations of lymphocytic cells in portal area were noticed, specially in group supplemented with vitamin A.

In group which treated with AFT and vitamin C, focal degeneration of hepatocytes was seen the hepatic cells appeared swollen with granular cytoplasm, moreover, scattered individual hepatic cell necrosis associated with lymphocytic infiltration were seen. Mild proliferation of small tiny bile ductules and interlobular connective tissue septa were observed in few cases. The hepatic blood vessels were engorged with blood.

In group which treated with (AFT + vitamin E), the liver showed occasional focal areas of vacuolar degeneration, in addition to accumulation of mononuclear cells in both hepatic parenchyma and portal area.

Liver sections showed diffused centilobular degeneration, with bile duct proliferation, hepatocytic regenerative activity, mild fatty changes, and severe liver sinusoidal congestion.

The livers of rats showed congestion of the hepatic and portal blood vessels. The hepatocytes were rather swollen with granular cytoplasm. In some areas, the hepatic cells undergo hydropic degeneration. Focal areas of necrosis accompanied by cellular infiltration, mainly macrophages and lymphocytes occurred. The portal triads showed

slight proliferation fibrous connective tissue and mononuclear cell infiltration. Bile duct hyperplasia were a constant lesion.

The liver parenchymal cells show a separation of the fibrillar and granular components of the nucleoi. Glycogen loss, RNA depletion, and proliferation of the endoplasmic reticulum all occur within the first day of dosing. Parenchymal cell mitosis is decreased dramatically within this first day. Over the next several days, the sequence of events is periportal necrosis of parenchymal cell, fatty infiltration of the remaining parenchymal cells and extensive bile duct cell proliferation. The parenchymal cells are considerably more susceptible to the toxic effects of AFB_1 than are the nonparenchymal Kuppffer and bile duct cells. Parenchymal cell proliferation is absent for the first few days after an acute dose of AFB_1, thereafter, mitoses occur in this cell population. The regeneration of the parenchymal cell population begins after the proliferation of the bile duct cells is well underway.

The microscopic examination of the liver of rats feed on aflatoxin revealed vacuolar and hydropic degeneration of the hepatocytes.

Pyknosis of the nuclei of some hepatic cells was also noticed. Hyperplasia of the bile ductal epithelium and fibrous connective tissue proliferation in the portal area with few mononuclear cellular aggregation were found.

3.6.2. Rats kidney:

Aflatoxin B_1 affects the kidney in the form of cloudy swelling of the epithelial lining of renal tubules with mild interstitial fibrosis and congestion.

The microscopical findings of the kidney of rats treated with $AFTB_1$ showed dilatation and congestion of renal blood vessels associated with interstitial hemorrhage in between the renal tubules in addition to perivascular round cell aggregations.

Occasionally, renal glomeruli showed hypercellularity of the glomerular. The epithelial lining of renal tubules showed necrobiotic changes and revealed marked increase in their size (megalocytosis) in some tubules, the nuclear enlargement was accompanied with presence of intranuclear inclusions.

In vitamin A and β-carotene supplemented groups moderate degenerative changes of epithelial lining the renal tubules were seen. Perivascular and periglomerular round cell aggregations were noticed. Minor degree of megalocytosis was observed in the proximal tubular epithelium. While in vitamin C group, the renal tubules showed mild degenerative changes. In vitamin E, supplemented group, some renal tubules revealed mild vacuolar degeneration. Meanwhile others, revealed cloudy swelling of the epithelium lining with subsequent narrowing of tubular lumina.

The major alterations in the kidneys were focal tubular cell necrosis, swelling of the glomeruli with hypercellularity and mesangial expansion.

The kidneys of rats showed congestion of glomerular capillaries and intertubular blood vessels. Tubulonephrosis and focal areas of mononuclear cell infiltration were observed in the inner cortex.

Homogenous eosinophilic casts were noticed in the lumens of the renal tubules.

Cloudy swelling of the lining epithelium of the renal tubules was seen. Disintegration and desquamation of the epithelium of some renal tubules were recorded. Moreover, coagulative necrosis of renal tubules evidenced by pyknosis of nuclei, loss of cell outlines and high eosinophilia of cytoplasm was also observed.

3.6.3. Rats heart:

Aflatoxins produced marked interstitial fibrosis. The myocardium revealed atrophy and necrosis.

3.6.4. Rats spleen:

Aflatoxin B_1 affects the spleen in the form of marked necrosis of red pulp with faint lymphoid follicles and large number of necrotic lymphocytes.

Slight lymphoid depletion was observed in the spleen.

3.6.5. Rats lung:

Aflatoxicosis causes lung congestion and haemorrhage, edema, thickened alveolar wall by inflammatory cells peribronchial fibrosis with thick-walled blood vessels. These are in accordance with the results of **Gopuiath et al. (1987)** who recorded that when rats were treated by intraperitoneal injection of the toxin, they showed pronounced inflammatory reaction in their lungs, congestion, destruction of alveolar walls with emphysematous changes and thickening of the walls of the blood vessels, occurring in patchy areas of the lung tissue.

The lung lesions consisted of peribronchial lymphoid hyperplasia associated with congestion of pulmonary vessels and alveolar wall

capillaries. Haemorrhages were sometimes seen in the alveoli, bronchi and bronchioles.

The histopathological examination of the lungs revealed accumulation of pink homogenous exudates mixed with mononuclear inflammatory cells inside the pulmonary alveoli.

Desquamations of the lining epithelium of the branchioles were prevalent. Thickening of blood vessel walls with perivascular lymphocytic cellular aggregation was seen. Moreover, focal areas of compensatory alveolar emphysema with lymphocytic cellular infiltration of the pulmonary tissues were also recorded.

3.6.6. Rats brain:

Focal area of encepholomalacia with neural degeneration, satellitosis and neurophagia were the main microscopic lesions observed in the brain of rat fed on aflatoxin.

3.7. Discussion of histology

3.7.1. Effect on body weight and some organs of rats.

The morphological and phathological lesions causes by aflatoxins in animals occur mostly in the liver. Focal lipid accumulation, parenchymal cell degeneration and necrosis, bile duct hyperplasia and hepatocellular carcinoma were commonly seen **(Bassir and Emafo, 1970; Bassir and Emerale, 1975)**.

The picture of hepatic lesions were mainly fatty change, perilobular fibrosis associated with necrosis and infiltration with lymphocytes and macrophages. Similar findings were obtained by

Mobarak et al. (1996) in Japanese quail, **Mohamed and Mokhbatly (1997)** in Tilapia. **Espada et al. (1992)** in chicken, **Gradelet et al. (1997)** in rats and **Netke et al. (1997)** in Guinea pigs.

The liver was the target organ for aflatoxin toxicity. Progressive hepatic degeneration, necrosis, bile duct hyperplasia and formation of many granulomas in the liver were commonly observed in animals of experiments. Similar lesions were described by other investigators **(Stevens et al., 1960; Bassir and Emerale, 1975)**.

Hyperplasia of bile duct cells is considered a specific response of aflatoxins **(Carnaghan et al., 1963; Wogan, 1965)**.

Lillehoj et al. (1970) attributed the liver lesions to the action of aflaotixn B_1 which interferes with DNA transcription and subsequently impaired synthesis of DAN and DNA-dependant RNA synthesis. Other lesions observed in other organs were variable. Congestion of glomerular capillaries and degeneration of tubular epithelium in the kidneys, haemorrhage, oedema, congestion and peribronchial lymphoid hyperplasia in the lung, and lymphoid depletion in the spleen were commonly observed.

Regarding, the effect of AFT on kidney, a significant increase in the weight and a non-significant increase in plasma urea and creatinine were recorded. This effect may be attributed to glomular and tubular affections that confirmed by histopathological findings of the present study. Such alterations were in parallel line with observations of **Rati et al. (1991)** and **Mobarak et al. (1996)** and could be due to direct nephrotoxic effect of AFT.

The results in Table (7) showed that their are a marked drop in the body weight of rats treated with AFT was observed. Similar results were reported by many investigators **(Brucato et al., 1986, Hendrickse, 1991)**. This drop may be due to decreased in the nitrogen absorbability in the diet as reported by **Reddy et al. (1991)** or loss of appetite and diarrhea **(Cook et al., 1989)** and the effect on liver capacity for protein synthesis **(Wogan, 1975)**.

The results showed significant decrease in liver and body weight ($P < 0.05$), while kidney weights were significantly increased ($P < 0.05$) in AFT treated group as compared with control group.

3.7.2. The control group (-ve aflatoxin):

The control group did not show abnormal changes during the study period. They continued to be healthy and very active. The microscopic characteristics of the liver and kidney sections were considered normal.

3.7.3. Group AFB_1 only

The average weights of the rats showed a dramatic decline after treatment with aflatoxin. At necropsy the liver and kidney in most of the rats showed enlargement. Liver sections showed diffused centrilobular degeneration, with bile duct proliferation, hepatocytic regenerative activity, mild fatty changes, and severe liver sinusoidal congestion. The major alterations in the kidneys were focal tubular cell necrosis, swelling of the glomeruli with hypercellularity and mesangial expansion.

3.7.4. Effect of supplementation the contaminated diet with vitamins on rats:

Supplementation with vitamins (especially vitamin C and E) leads to increased body and liver weight, reduced activities of ALP, ALT and

AST with mild histopathological changes. The protective activity of ascorbic acid was due to induction of enzyme $AFTB_1$-epioxide hydrolase or aldehyde reductase or both **(Netke et al., 1997)**. Moreover, vitamin E protect cells from damage by conjugating $AFTB_1$-epoxide shortly after their formation thus preventing these reactive metabolites from binding to essential cellular protein or nucleic acids, with subsequent reduction in AFT-metabolism **(Brucato et al. 1986 and Ibeh and Saxena, 1998)**.

A non significant increase in body weight was recorded in groups supplemented with vitamins (A), β-carotene, and E, moreover, significant increase in liver weight after supplementation with vit. E was recorded. The kidney weights of rats treated with vitamin A, β-carotene and vitamin C showed significant decrease.