RESULTS

4.1. Fungi associated with corn (*Zea mayes-L*) samples:

The fungi associated with corn grains were studied using the two tested standard methods of corn grains (i.e. blotter and agar plate). In Blotter test, the disinfected corn grain samples collected from Kafr El-Sheikh governorate recorded the higher total fungal count whereas, Gharbia governorate recorded the lowest total fungal count (Table 1). On the other hand, all the corn grain collected from Gharbia governorate germinated and the corn grain collected from Kafr El-Sheikh showed the lower germinated number of the tested grain (Table 1). The percentage of total fungal count and germination are presented in Table (1) and Fig. (9). Forty six percent of the corn grain samples collected from Kafr El-Sheikh governorate were infected with fungi and the percentage of germination of these corn grains was the lowest (32%) compared to the other governorates. Whereas, corn grain samples collected from Gharbia governorate recorded the lowest total fungal count and the higher percentage of germination (100%).

Table (1). Total and percentage of fungal counts and germination of disinfected corn grain collected from different governorate in Egypt as determined by Blotter test.

Governorate	Total fungal count	%	Total Germination	%
B. S	13	23	40	71
GH	0	0	56	100
K. E	26	46	18	32
Q	17	30	43	77
SH	23	41	43	77

B. S: Bany-Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

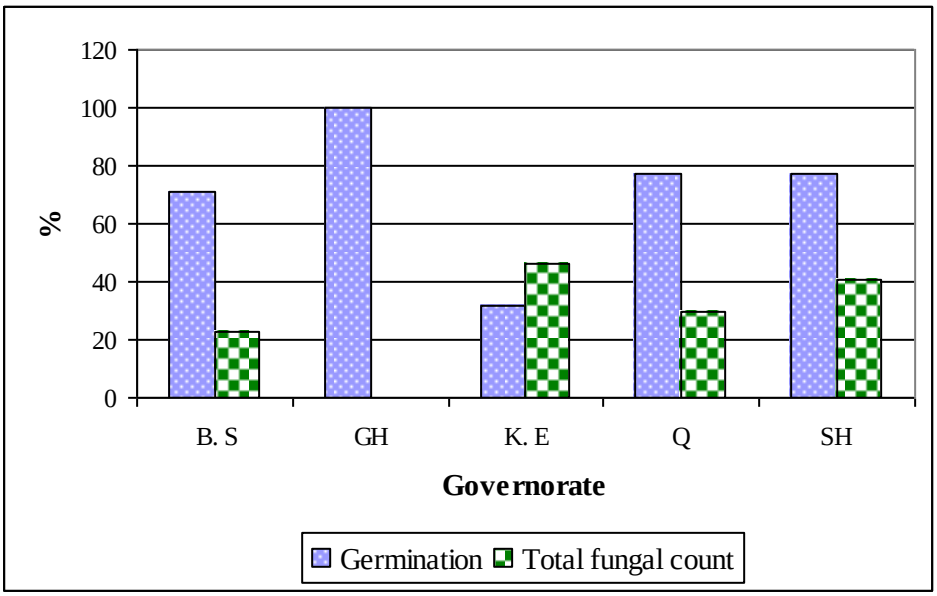


Fig. (9). Percentage of total fungal counts and germination in disinfected corn grain collected from different governorate determined by Blotter test

The results of the total fungal count and germination of non-disinfected corn grain determined by Blotter test are presented in Table (2). It is clear that

corn grain samples collected from Kafr El-Sheikh recorded the higher total fungal count and the lower germination rate compared to the other samples collected from the other governorates (Table 2). Whereas, no fungi were isolated from corn grain samples collected from Gharbia. Consequently, these all corn grain were germinated and recorded 100% of germination (Fig. (10).

Table (2). Total and percentage of fungal counts and germination of non-disinfected corn grain collected from different governorate in Egypt as determined by Blotter test.

Governorate	Total fungal count	%	Total Germination	%
B. S	27	48	40	71
GH	0	0	56	100
K. E	44	79	13	23
Q	23	41	40	71
SH	27	48	34	61

B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

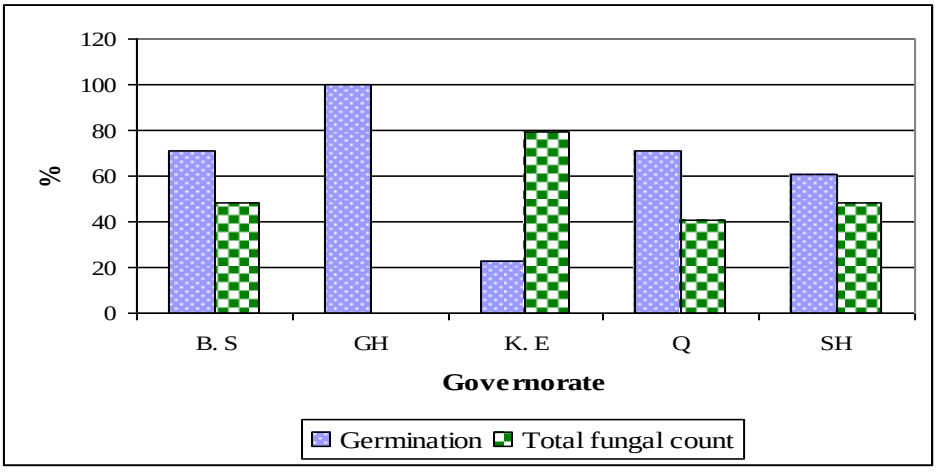


Fig. (10). Percentage of total fungal counts and germination in non-disinfected corn grain collected from different governorate determined by Blotter test.

In PDA test, the total fungal count in disinfected corn grain samples collected from Kafr El-Sheikh recorded the higher fungal count whereas, the

samples collected from Gharbia recorded the lowest fungal counts (Table 3). The disinfections of the tested corn grain seeds lead to higher percentage of germination (Fig. 11). It is clear that 100% of the corn grain samples collected from Gharbia were germinated while only 39% of the corn grains sample collected from Kafr El-Sheikh were germinated. It is of interest to mention that all the corn grain samples (100%) collected from Sharkia were germinated although total fungal count recorded 39% of these grains.

Table (3). Total and percentage of fungal counts and germination of disinfected corn grain collected from different governorate in Egypt as determined by PDA test.

Governorate	Total fungal count	%	Total Germination	%
B. S	25	45	54	96
GH	17	32	56	100
K. E	45	80	22	39
Q	34	61	46	82
SH	36	64	56	100

B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

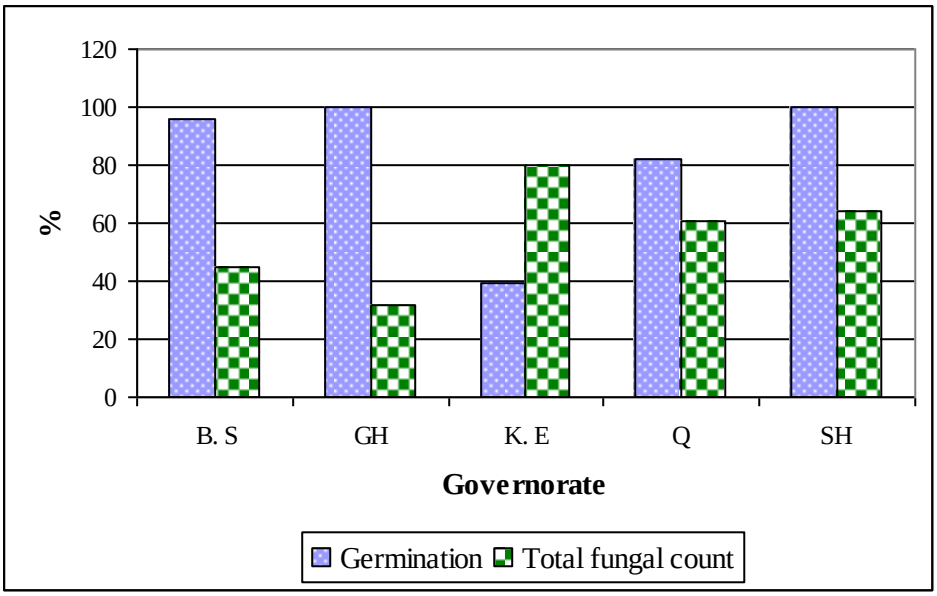


Fig. (11). Percentage of total fungal counts and germination in disinfected corn grain collected from different governorate determined by PDA test.

The results of total fungal counts and germination of non-disinfected corn grain collected from different governorates carried out by PDA test are presented in Table (4). These results indicated that corn samples collected from Kafr El-Sheikh governorate recorded the higher total fungal count and germination rate followed by the corn samples collected from Sharkia. However, Gharbia recorded the lowest total fungal counts and higher germination rate. The percentage of total fungal counts and germination of disinfected corn grain determined by PDA test are presented in Fig (12). It is clear percentage of TFC in corn grain samples collected from Kafr El-Sheikh was 95% however; it recorded 33% for Gharbia governorate. On the other hand, percentages of germination of corn grain for the same governorates were 100% and 34% respectively.

Table (4). Total and percentage of fungal counts and germination of non-disinfected corn grain collected from different governorate in Egypt as determined by PDA test.

Governorate	Total fungal count	%	Total Germination	%
B. S	30	54	54	96
GH	18	33	56	100
K. E	53	95	19	34
Q	38	68	36	64
SH	47	82	53	95

B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

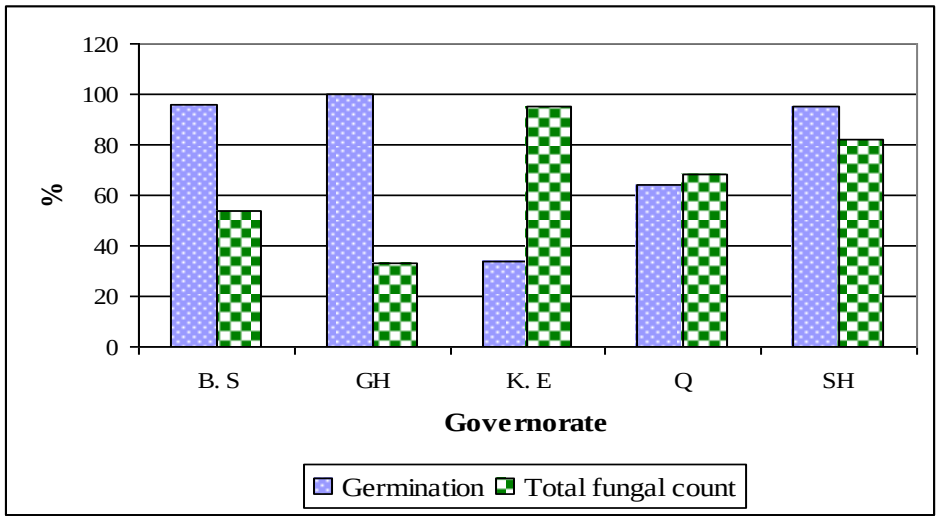


Fig. (12). Percentage of total fungal counts and germination in non-disinfected corn grain collected from different governorate determined by PDA test

Data presented in Fig (13) showed that disinfected and non-disinfected corn grains collected from Bany-Swief recorded 96% of germination in either blotter test or PDA medium. Whereas, corn grain collected from Qualubia recorded 82% and 64% germination for disinfected and non-disinfected

seeds respectively in PDA medium, however; it recorded 77% and 71% in blotter test for disinfected and non-disinfected seeds respectively. On the other hand, results in the same data (Fig 13) indicated that disinfected corn grain seeds were less in infection and in colony percentage than non-disinfected seeds in all governorate samples. Moreover, the same data showed that agar plates (PDA) was found to be an enhanced method and gave higher numbers of fungal colony associated with all tested governorates samples compared to the blotter test method. The percentage of colony and the numbers of fungi on agar plate (PDA) method were 45% and 54% for Bany-Swief, 32% and 33% for Gharbia, 80% and 95% for Kafr-El-Shikh and 64% and 84% for Sharkia governorate in disinfected and non-disinfected corn grain samples respectively, while blotter test method gave the lowest numbers of fungal colony which recorded 23% and 48% for Bany-Swief, zero percent and zero percent for Gharbia, 46% and 79% for Kafr El-Shikh, 30% and 41% for Qualubia and 41% and 48% for disinfected and non-disinfected corn grain samples respectively.

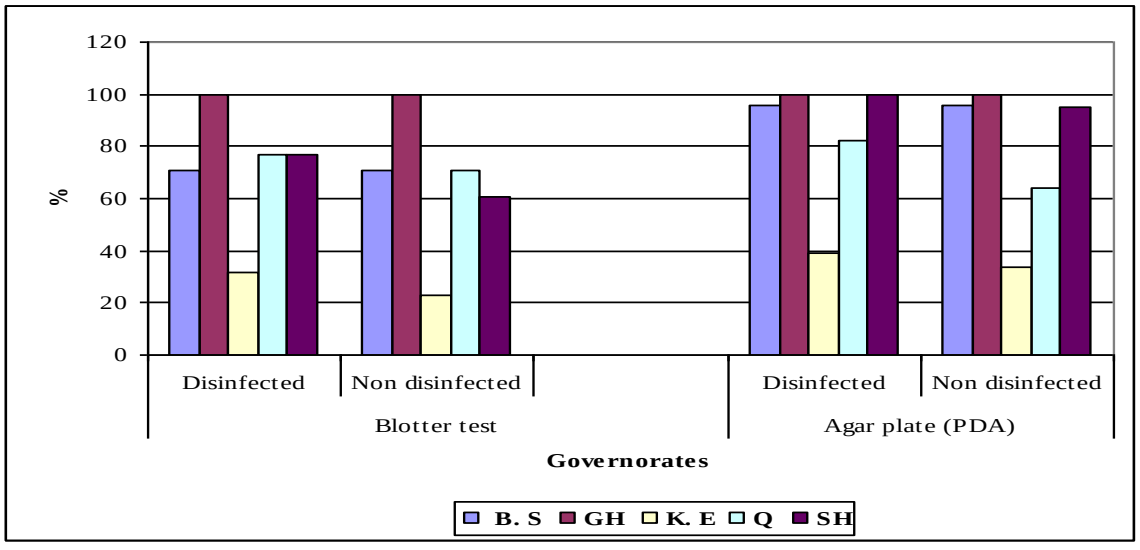


Fig. (13). Percentage of germination in disinfected and non-disinfected corn grain samples collected form different Egyptian governorates.

4.2. Microorganisms associated with corn grain samples:

The isolation of fungi from corn grain samples collected from the five tested governorates resulted in 1580 total fungal isolates. Isolation of fungi using blotter test resulted in 780 isolates (Table 5) whereas, isolation using agar plate (PDA) test resulted in 800 isolates (Table 6). Four major fungal genera were isolated and were identified as *Aspergillus, Fusarium, Penicillium* and *Trichoderma.* Moreover, these data showed that *Penicillium spp* was the most frequency occurred (600) in all tested of corn grain samples within all tested governorates. Whereas, *Fusarium oxysporum* and *Asp. ochrecus* were found to be the lesser frequency of fungal isolates and gave (10). Moreover, *Fusarium* was found to be associated only with non-disinfected corn grain of Kafr El-Shikh samples and *Asp. ochrecus* was found to be associated with only non-disinfected of corn grain Sharkia samples. Taken together, data presented in Tables (5 and 6) showed that corn grain samples collected form Bany Swief governorate represent the most fungal frequency compare to the other governorates. These samples gave 430 isolates followed by Kafr El-shikh, Qualubia and Sharkia respectively, however; Gharbia was found the lesser governorate regarding the occurrence of fungal frequency.

Table (5). Fungal frequency isolated from corn grain samples collected from different governorates of using blotter test.

Gover. / Species	B. S				GH				K. E				Q				SH				Total	
	D		N		D		N		D		N		D		N		D		N			
	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%
Asp. flavus	10	1.3	30	3.8	--	--	--	--	--	--	--	--	10	1.3	--	--	50	6.4	80	10.2	180	23
Asp. niger	10	1.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	20	2.6	10	1.3	40	5..2
Asp. ochrecus	--	--	--	--	--	--	--	--	--	--	--	---	--	--	--	--	10	1.3	--	0.0	10	1.3
Fusarim Oxysporum	--	--	--	--	--	--	--	--	--	--	10	1.3	--	--	--	--	--	--	--	--	10	1.3
Penicillium spp.	110	14.1	20	2.6	--	--	--	--	--	--	--	--	90	11.5	20	2.6	--	--	10	1.3	25	32.1
Trichoderma spp.	--	--	20	2.6	--	--	--	--	240	30.7	10	1.3	20	2.6	--	--	--	--	--	--	29	37.1
Total	130	16.7	70	9	--	--	--	--	240	30.7	20	2.6	120	15.3	20	2.6	80	10.3	100	12.8	780	100

Where -- = free of fungi D = Disinfect N = Non disinfect T.C = Total count % = Percentage of infection.

B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

Table (6). Fungal frequency isolated from corn grain samples collected from different governorates using PDA test:

Gover. / Species	B. S				GH				K. E				Q				SH				Total	
	D		N		D		N		D		N		D		N		D		N			
	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%	T.C	%
Asp. flavus	40	5	--	--	--	--	--	--	--	--	--	--	20	2..5	--	--	70	8.8	20	2..5	150	18.8
Asp. Niger	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	20	2.5	--	--	20	2..5
Asp. Ochrecus	--	--	--	--	--	--	--	--	--	--	--	---	--	--	--	--	--	--	--	--	--	--
Fusarim Oxysporum	--	--	--	--	--	--	--	--	--	--	--		--	--	--	--	--	--	--	--	--	--
Penicillium spp.	90	11.3	100	12.5	--	--	30	3.7	--	--	10	1..2	40	5	60	7..5	20	2..5	--		350	43.7
Trichodera spp.	--	--	--	--	--	--	20	2..5	80	10	60	7..5	40	5	60	7..5	--	--	20	2..5	280	35
Total	130	16.3	100	12.5	--	--	50	6..2	80	10	70	8.7	100	12.5	120	15	110	13.8	40	5	800	100

Where -- = free of fungi D = Disinfect N = Non disinfect T.C = Total count % = Percentage of infection.

B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

4.3. Mycotoxin production:

The isolated fungi fungal genera e.g. *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* were tested for their mycotoxins production using thin layer chromatography (TLC) method. Data presented in Table (7) indicated that *Aspergillus flavus* isolated from Sharkia corn grain samples was producer of aflatoxins, while the other genera isolated from the other governorates samples were not able to produce aflatoxins. *A. flavus* isolated from Sharkia governorate samples (isolate no. 208) was further tested for their production of aflatoxins using high performance liquid chromatography technique (HPLC). The results indicated that this isolate has the ability to produce aflatoxin B_1 and B_2 (Table 8).

Table (7). Mycotoxin production by fungi isolated from corn grain samples from different governorats.

Governorate / Species	B. S			GH			K. E			Q			SH		
	AF	FB	OA	AF	FB	OA	AF	FB	OA	AF	FB	OA	AF	FB	OA
Asp. flavus	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Asp. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asp. ochrecus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium Oxysporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Where AF = Aflatoxins FB = Fumonisins OA= Ochratoxin A
B. S: Bany Swief; GH: Gharbia; K.E: Kafr El-Shiekh; Q: Qualubia; SH: Sharkia

The HPLC quantity determination of aflatoxins produced by *A. flavus* (isolate no. 208) showed that this isolate produce 1.9742 µg/g corn grains aflatoxin B_1 (1974.2 µg/kg or 197400 µg/Ton) and 0.01518 µg/g corn grains aflatoxin B_2

(15.18 µg/kg or 15180 µg/Ton) when artificially inculcated and incubated for 2 weeks under laboratory condition (Table 8 and Fig 14).

Table (8). Concentration of aflatoxins produced by *A. flavus* (isolate no. 208) isolated form Sharkia governorate samples.

Type of aflatoxin	µg/g	µg/kg	µg/Ton
AFB_1	1.9742	1974.2	197400
AFG_1	ND	ND	ND
AFB_2	0.01518	15.18	15180
AFG_2	ND	ND	ND

Where ND = Not detected

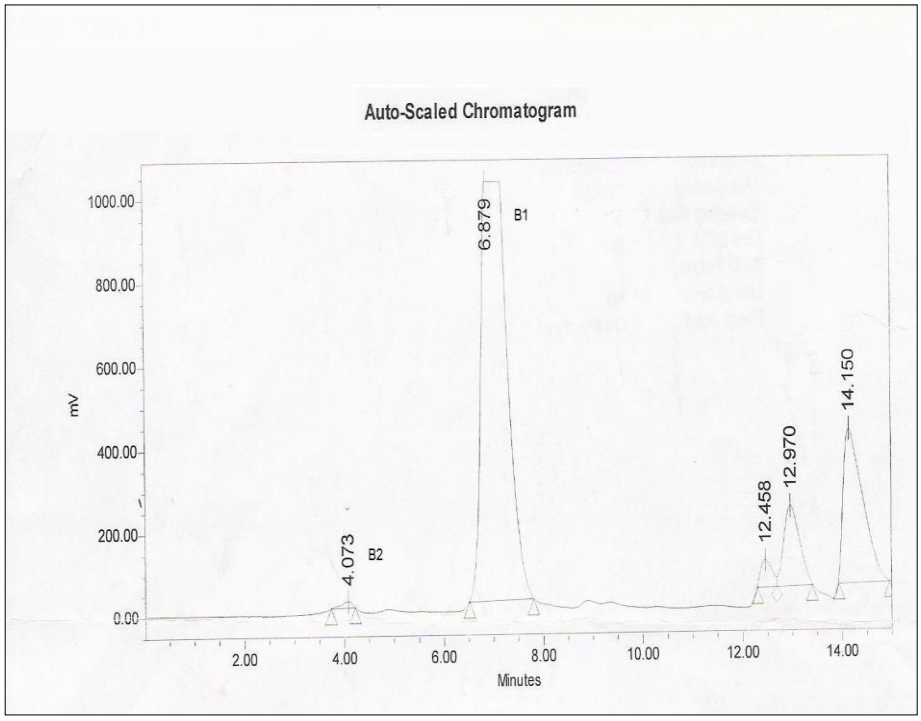


Fig (14). Aflatoxins concentration produced by *A. flavus* (isolate no. 208) and determined by HPLC.

Table (9). Chemical composition of uncontaminated and contaminated corn grains.

Component	corn grains (%)	Contaminated corn grains (%)
Lipid	**3.920**	**3.001**
Protein	**12.511**	**9.933**
***Carbohydrate**	**65.813**	**47.401**
Ash	**1.852**	**1.710**
Moisture	**13.801**	**36.043**
Fiber	**2.103**	**1.912**

Where * = Carbohydrate calculated by difference.

Data presented in Table (9) revealed that the composition of non-infected corn grain contain lipid (3.920%), protein (12.511%), carbohydrate (65.813%), ash (1.852%), moisture (13.801%) and fiber (2.103). On the other hand, the chemical composition of the corn infected with *A. flavus* revealed that the fungal infection resulted in the decrease of all chemical composition of the corn grain. The obtained data (Table 9) indicated that the lipid content was 3.001%, the protein content was 9.933%, the carbohydrate was 47.401%, the ash was 1.710%, the moisture was 36.043% and the fiber was 1.912%. Generally the obtained results suggested that the decrease in the chemical composition reached 23.44% in lipid content, 20.6% in protein content, 27.976% in carbohydrate content and 9.08% in fiber content. However the moisture content was found to be increased by 161.16%.

4.4. Antifungal effects of *Aquilegia vulgaris* L extract:

The effects of *Aquilegia vulgaris* L extract on growth rate (mm) and inhibition zone (mm) of *A. flavus* are presented in Table (10). It is clear that the *A. vulgaris* L extract significantly reduced the growth rate of *A. flavus.* Moreover, the growth rate of fungus was decreased with increasing the concentration of the plant extract (Fig. 15). Fig (16) is a photograph for the control plate of *A. flavus*

indicating the normal growth rate of the fungi. The percentage of growth inhibition of *A. flavus* reached 8.23%, 11.7% and 16.47% when the extract was used at concentrations of 0.5 ml (Fig. 17), 1.0 ml (Fig. 18) and 1.5 ml/plate (Fig. 19) respectively. Furthermore, the results of inhibition zone (Table 10) revealed that the inhibition zones were increased with increasing the application of plant extract and reached 7.0 mm, 10 mm and 14 mm with the three tested plant extract concentration (Fig 20).

Table (10). Effect of *Aquilegia vulgaris-L* extract on growth rate (mm) of *A. flavus.*

Conc.	U	T		Red. %
	Control(mm)	In(mm)	G (mm)	
0.5 ml	85	7	78	8.23
1.0 ml	85	10	75	11.76
1.5 ml	85	14	71	16.47

Where Conc. = Concentration U = Untreated T = Treated
In = Inhibition zone G = Growth rate Red = Reduction

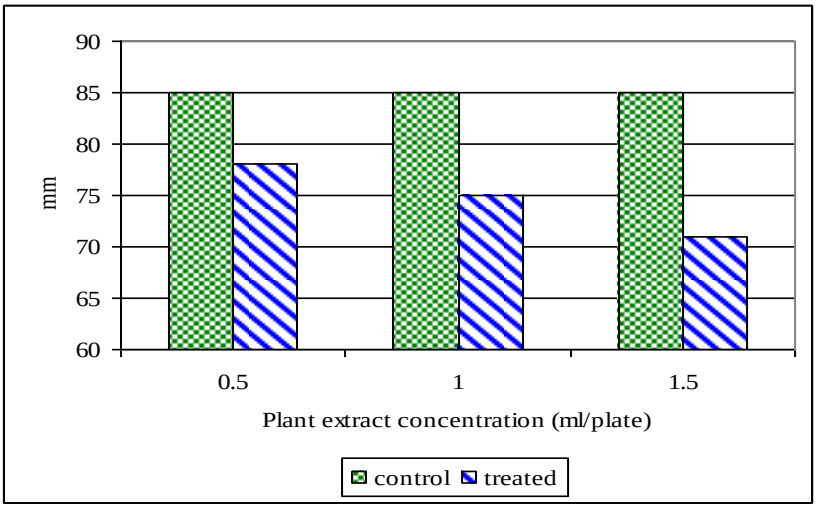


Fig (15). Growth rate for control and plant extract-treated *A. flavus*

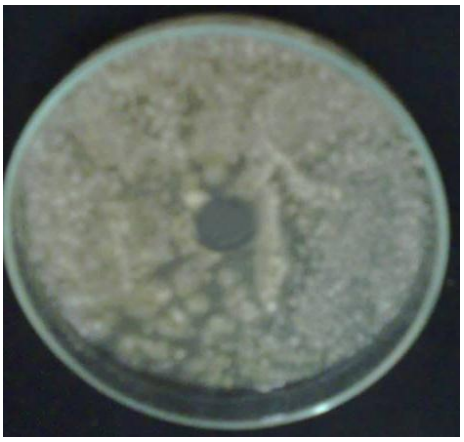


Fig. (16). Growth rate of *A. flavus* isolated from corn grains

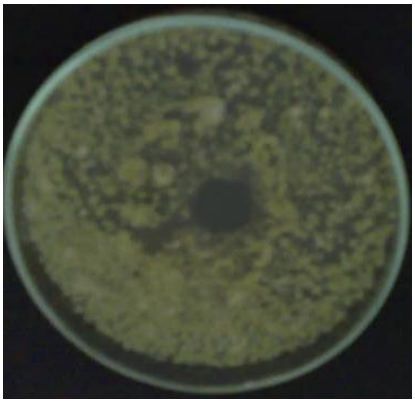


Fig. (17). Growth rate and inhibition zone of *A. flavus* treated with 0.5 ml of *Aquilegia vulgaris-L* extract

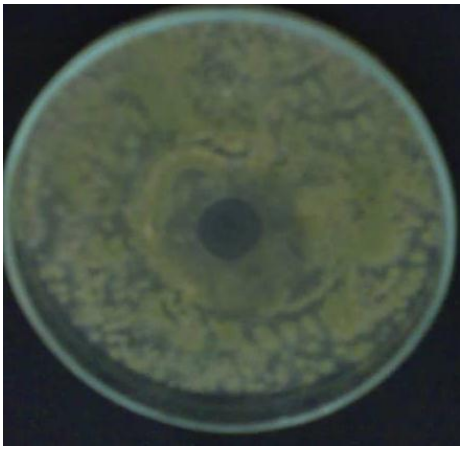
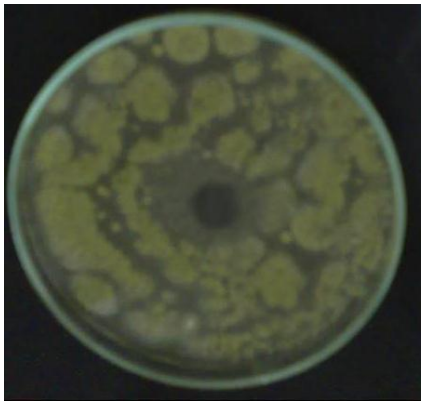


Fig. (18). Growth rate and inhibition zone of *A. flavus* treated with 1.0 ml of *Aquilegia vulgaris-L* extract



Fug. (19). Growth rate and inhibition zone of *A. flavus* treated with 1.5 ml of *Aquilegia vulgaris-L* extract

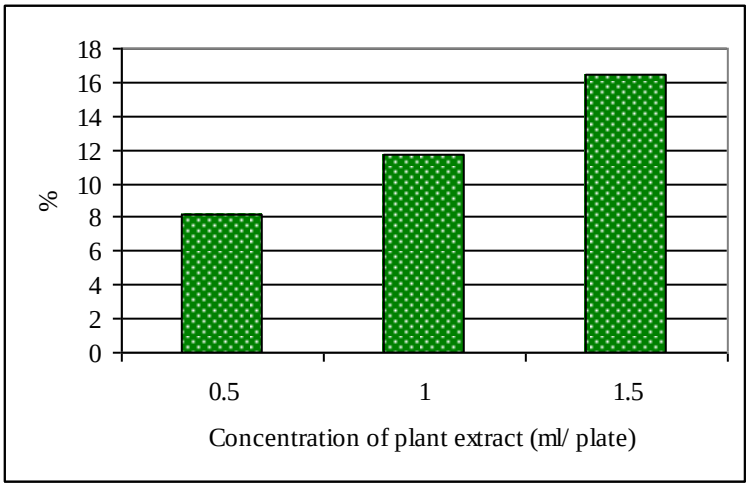


Fig (20). Inhibition percentage of *A. falvus* treated with different concentration of *Aquilegia vulgaris-L* extract

Biological evaluation of the *Aquilegia vulgaris -L* extract:

The *Aquilegia vulgaris -L* extract was further evaluated for its protective effect against aflatoxin toxicity in rats as a sensitive model of laboratory animals. The results of the current study presented in Table (11) revealed that animal fed aflatoxin-contaminated diet showed significant increase in AST (Fig 21), ALT (Fig 22) and ALP (Fig 23) activity. Analysis of variance (Table 12) indicated that there is a significant difference between different treatment groups in liver function enzymes activity. Animal received the plant extract alone were comparable to the control in AST, ALT and ALP. On the other hand, animals fed aflatoxin-contaminated diet and were administrated the plant extract showed a significant improvement in these biochemical parameters of liver function. These animals were also comparable to the controls.

Table (11). Effect of *Aquilegia vulgaris -L* extract on AST, ALT and ALP in rats fed aflatoxin-contaminated diet (2 mg/kg diet).

Parameters / Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**
Control	149.3 ± 5.1[a]	53.3 ± 3.2[a]	188.1 ± 12.2[a]
Aflatoxin	177.2 ± 3.2[b]	82.1 ± 2.5[b]	258.8 ± 10.6[b]
Extract	145.5 ± 5.1[a]	55.2 ± 2.2[a]	191.9 ± 9.8[a]
Aflatoxin + Extract	154.4 ± 10.2[a]	60.4 ± 2.2[a]	229.5 ± 15.6[a]

Within each column, means superscript with different letter are significantly different ($P \leq 0.05$).

Data are expressed as means ± S.E.

Table (12). ANOVA for the difference between different treatment groups in AST, ALT and ALP.

S.O.V	df	MS		
		AST	ALT	ALP
Between groups	3	2019*	1754.2*	11229
Within groups	36	418	66.47	1498.5
Total	39			

MS: mean squares
df: degrees of freedoms
* significant at P≤ 0.05

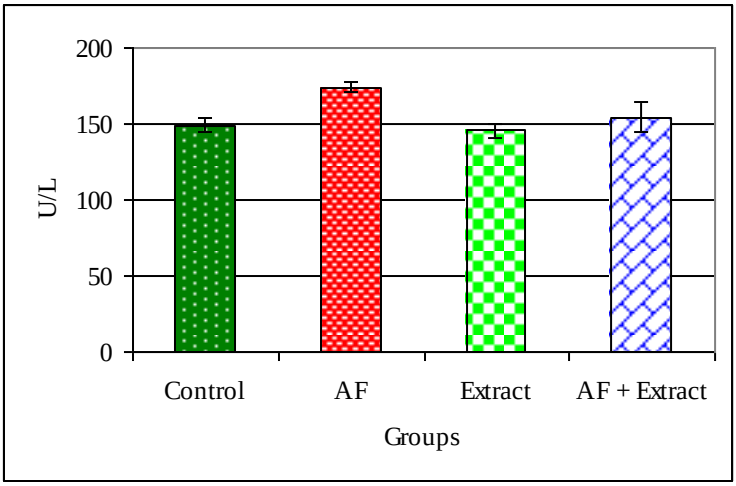


Fig (21).Changes in AST activities in rats fed aflatoxin contaminated diet with or without *Aquilegia vulgaris -L* extract.

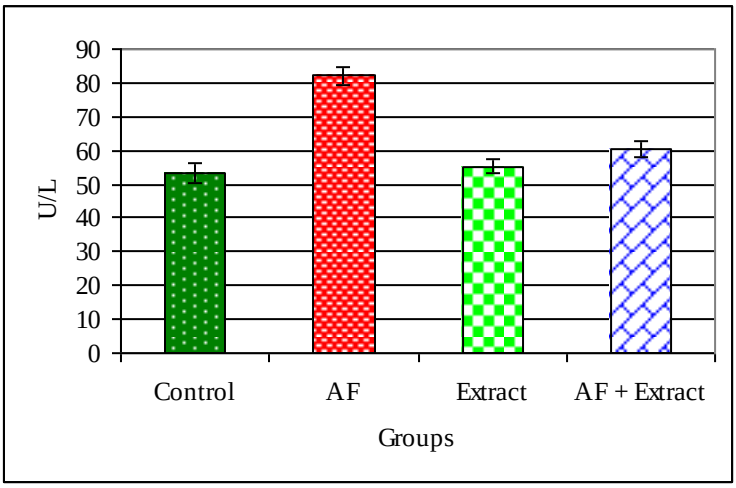


Fig. (22). Changes in ALT activities in rats fed aflatoxin contaminated diet with or without *Aquilegia vulgaris -L* extract.

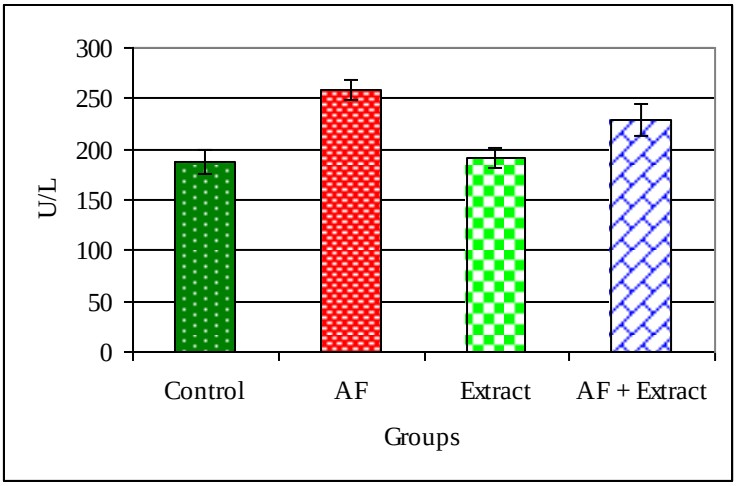


Fig. (23). Changes in ALP activities in rats fed aflatoxin contaminated diet with or without *Aquilegia vulgaris -L* extract.

The effects of different treatments on kidney function are presented in Table (13). It is clear that aflatoxin has nephrotoxic effects as demonstrated by the significant increase in serum urea (Fig. 24), createnine (Fig. 25) and uric acid (Fig. 26). Analysis of variance (Table 14) revealed that there is a significant difference between the animal within different treatment groups in kidney function tests.

On the other hand the same data revealed that animals treated with the *Aquilegia vulgaris -L* extract alone were comparable to the control. The combined treatment with aflatoxin and the *Aquilegia vulgaris -L* extract succeeded to reduce the elevation in kidney function parameters although these parameters were still insignificantly higher than the controls.

Table (13). Effect of *Aquilegia vulgaris -L* extract on kidney function of rats fed aflatoxin contaminated diet.

Parameters / Groups	Urea (mg/dl)	Createnine (mg/dl)	Uric acid (mg/dl)
Control	109 ± 4.7^{a}	0.64 ± 0.04^{a}	2.3 ± 0.1^{a}
Aflatoxin	144.6 ± 6.6^{b}	1.2 ± 0.1^{b}	3.2 ± 0.3^{a}
Extract	107.5 ± 4.7^{a}	0.67 ± 0.02^{a}	2.1 ± 0.1^{a}
Aflatoxin + Extract	108 ± 4.9^{a}	0.6 ± 0.04^{a}	2.2 ± 0.2^{a}

Within each column, means superscript with different letter are significantly different ($P \leq 0.05$)
Data are expressed as means ± S.E.

Table (14). ANOVA for the difference between different treatment groups in urea, createnine and uric acid.

S.O.V	df	MS		
		Urea	Createnine	Uric acid
Between groups	3	3322	0.8	2.7
Within groups	36	282	0.06	0.35
Total	39			

MS: mean squares
df: degrees of freedoms
* significant at $P \leq 0.05$

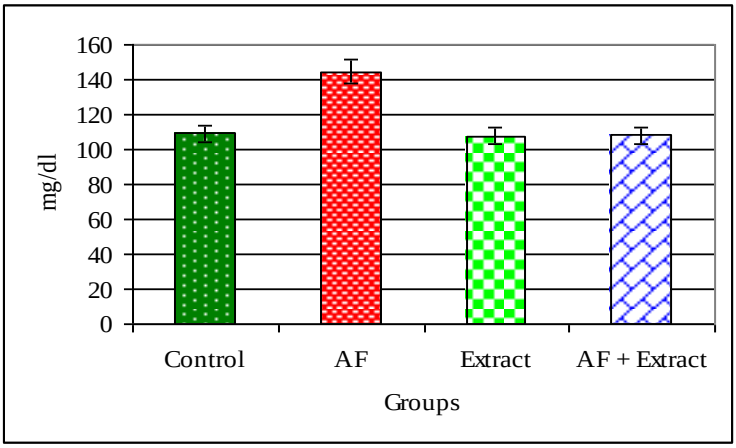


Fig. (24). Effect of *Aquilegia vulgaris -L* extract on serum urea in rats fed aflatoxin-contaminated diet

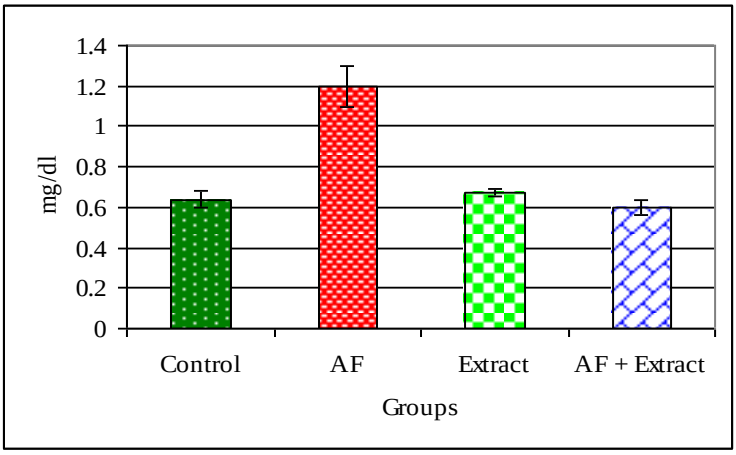


Fig. (25).Effect of *Aquilegia vulgaris -L* extract on serum createnine in rats fed aflatoxin-contaminated diet

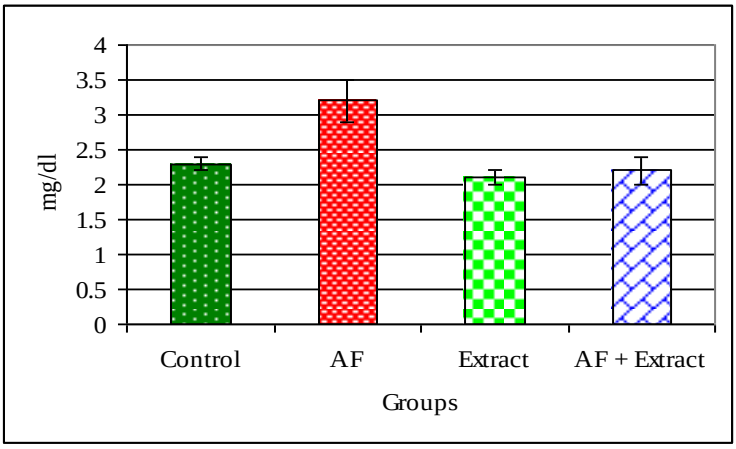


Fig. (26).Effect of *Aquilegia vulgaris -L* extract on serum uric acid in rats fed aflatoxin-contaminated diet

The current study revealed that aflatoxin has stressful effects on cardiac and muscle tissues as indicated by the significant increase in LDH and CK activities (Table 15). Analysis of variance (Table 16) indicated that a significant difference was found between treated animals within different groups in LDH and CK. Moreover, no significant difference was found in LDH or CK in animals treated with the extract alone. Animals treated with the extract plus aflatoxin showed a significant decrease in LDH blew the normal value of the control (Fig 27) whereas, CK activity was found to decrease to the normal level of the control (Fig.28).

Table (15). Effect of *Aquilegia vulgaris -L* extract on CK and LDH enzyme activities of rats fed aflatoxin contaminated diet.

Parameters / Groups	LDH (U/L)	CK (U/L)
Control	3072.5 ± 182[a]	228.7 ± 10.7[a]
Aflatoxin	3749.2 ± 224.9b	323.4 ± 14.7[b]
Extract	2880.4 ± 279.6[a]	226.3 ± 10.7[a]
Aflatoxin + Extract	2214.2± 254.3[c]	223.8 ± 14.8[a]

Within each column, means superscript with different letter are significantly different ($P \leq 0.05$)
Data are expressed as means ± S.E.

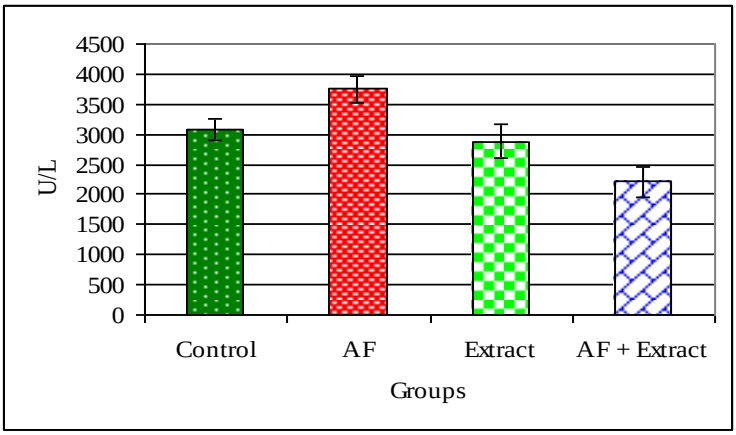


Fig. (27).Effect of *Aquilegia vulgaris -L* extract on LDH activity in rats fed aflatoxin-contaminated diet

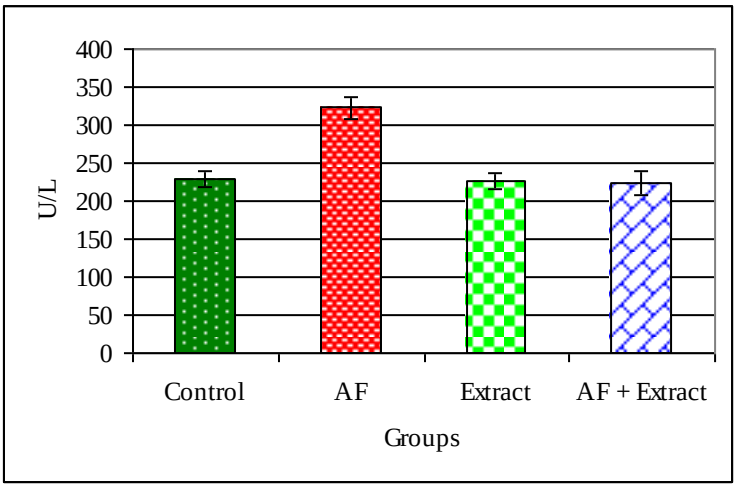


Fig. (28).Effect of *Aquilegia vulgaris -L* extract on CK activity in rats fed aflatoxin-contaminated diet

Table (16). ANOVA for the difference between different treatment groups in LDH, CK and AFP.

S.O.V	df	MS		
		LDH	CK	AFP
Between groups	3	23627	3988637.8	34.7
Within groups	36	1664	566352	0.46
Total	39			

MS: mean squares
df: degrees of freedoms
* significant at P≤ 0.05

The effect of different treatment on tumor marker in animals fed aflatoxin-contaminated diet or those treated with the extract alone or plus aflatoxin is illustrated in Fig (29). The data revealed that aflatoxin caused a significant increase in AFP activity (Table 15). Animals treated with extract alone were comparable to the control whereas, animal received the combined treatment of aflatoxin and the extract showed a significant improvement in AFP level although this treatment failed to normalize AFP since it was still significantly higher than the control value.

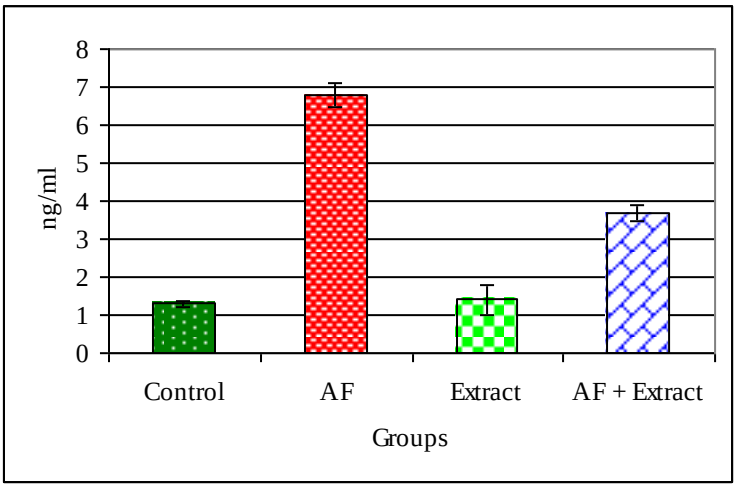
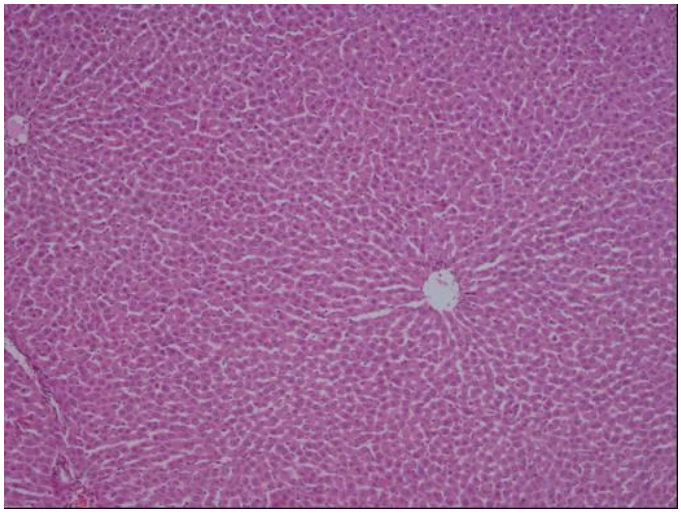


Fig. (29). Effect of *Aquilegia vulgaris -L* extract on AFP activity in rats fed aflatoxin-contaminated diet

The histological study of the liver revealed that the liver section of the control rats showed the general normal hepatocytes structure (Fig. 30). The hepatocytes of the liver in this group were arranged; the blood sinusoids and the central vein appeared normally (Fig. 31). The liver sections of the group fed aflatoxin-contaminated diet showed portal tract dilatation with hemorrhage, coagulative necrosis, thick walled and necrosis in hepatocytes around. Moreover, hyperplasia and hypertrophy in bile ducts also noticed (Fig. 32). The same sections in this group showed that the hypertrophy of bile duct epithelial cells with marked layer of connective tissue and inflammatory cells, thick walled, necrosis in hepatocytes around, the hyperplasia and hypertrophy in bile ducts were also noticed (Fig. 33). Moreover, other sections showed abnormal nuclear shape and size and hyper chromatin and eosinophilic as well as some hepatocytes showed nuclear desintigration (Fig. 34).

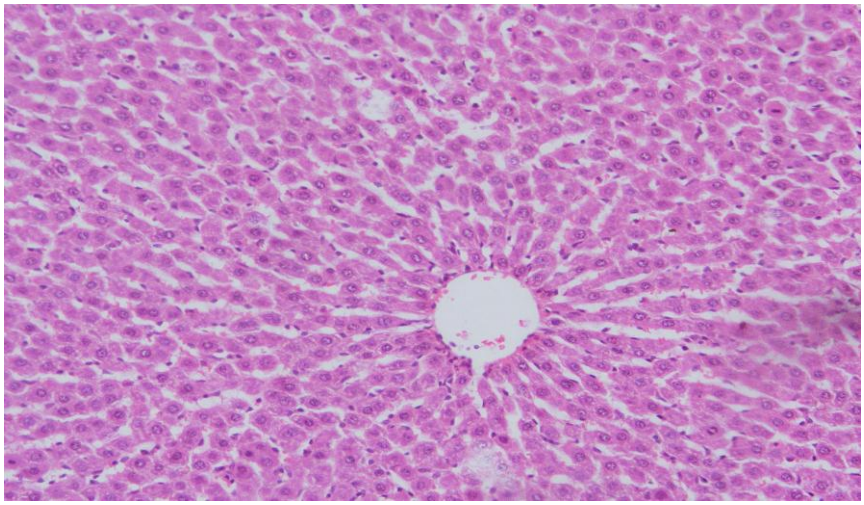
The liver sections of the animals treated with the extract alone showed normal hepatocytes structure (Fig. 35) and normal hepatocytes and nuclei containing one or two nucleolus (Fig. 36). The liver sections of the animal fed aflatoxin-

contaminated diet and treated with the extract showed regeneration of normal hepatocytes structure (Fig. 37) and normal hepatocytes, nuclei and central vein (Fig. 38).



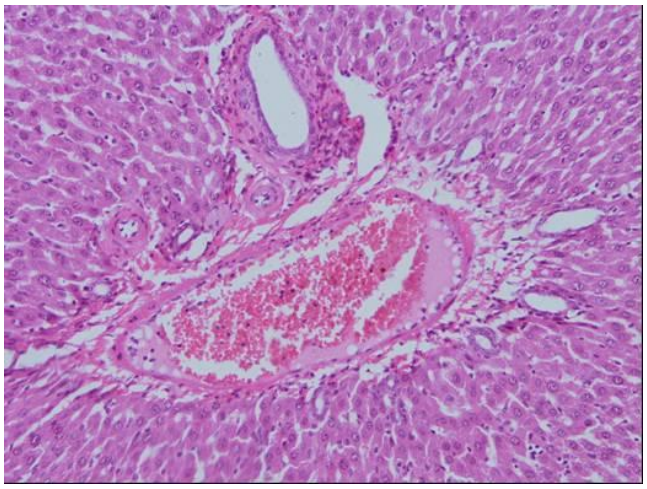
(Fig. 30). A photomicrograph in liver section of control rat liver showing the general normal hepatocytes structure.

(HX & E X 200)



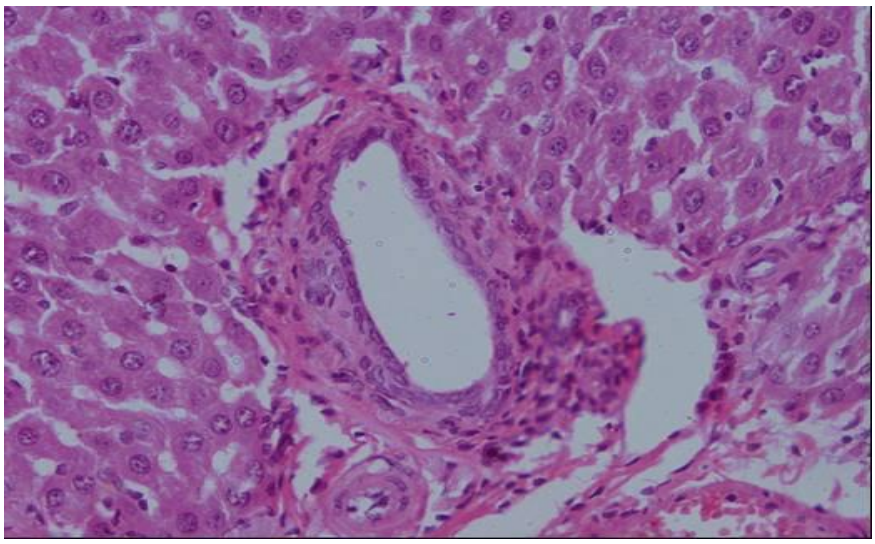
(Fig. 31). High magnification in a section of control rat liver showing the arranged hepatocytes, blood sinusoids and central vein.

(HX & E X 200)



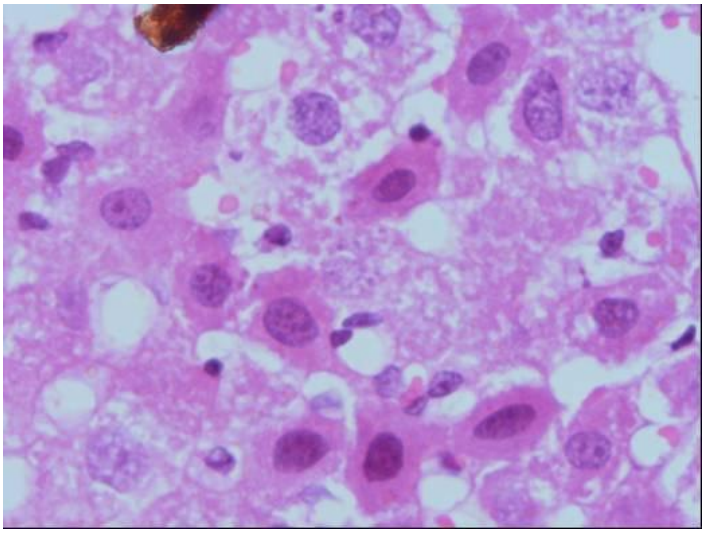
(Fig. 32). A photomicrograph in a liver section of rat fed aflatoxin-contaminated diet showing the portal tract dilatation with hemorrhage, coagulative necrosis, thick walled, necrosis in hepatocytes around. Hyperplasia and hypertrophy in bile ducts also noticed.

(HX & E X 200)



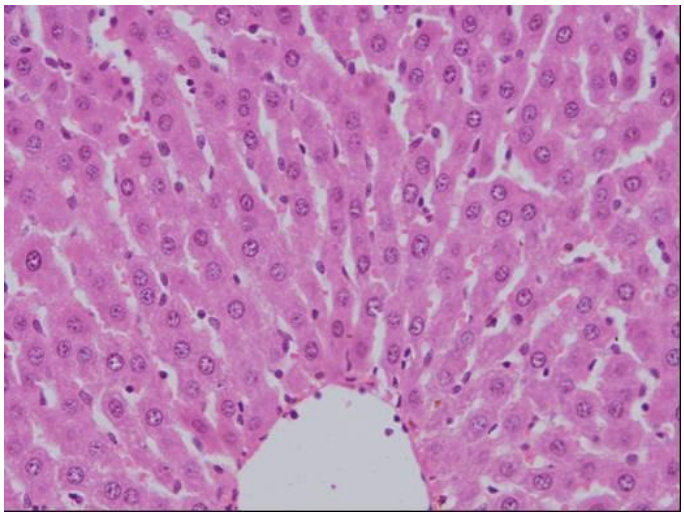
(Fig. 33). A photomicrograph in a liver section of rat fed aflatoxin-contaminated diet showing the hypertrophy of bile duct epithelial cells with marked layer of connective tissue, inflammatory cells, thick walled, necrosis in hepatocytes around. Hyperplasia and hypertrophy in bile ducts also noticed.

(HX & E X 400)



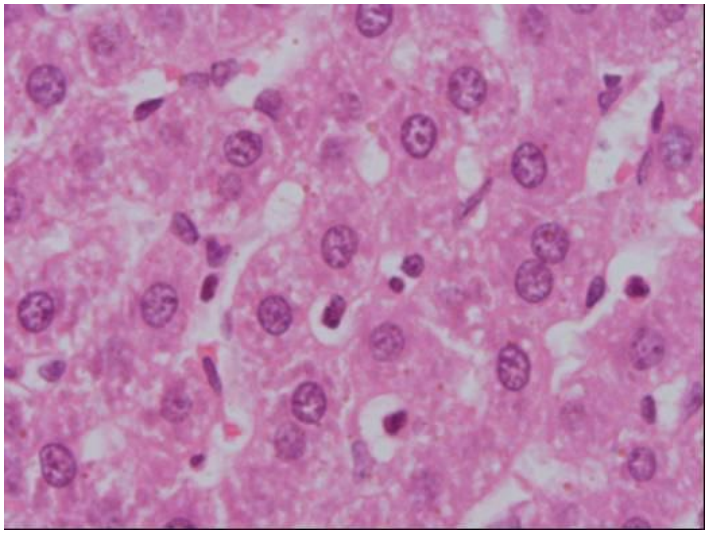
(Fig. 34). Another photomicrograph in a liver section of rat fed aflatoxin-contaminated diet showing the abnormal nuclear shape and size and hyper chromatin and eosinophilic. Some hepatocytes showing nuclear desintigration.

(HX&E X ١٠٠)



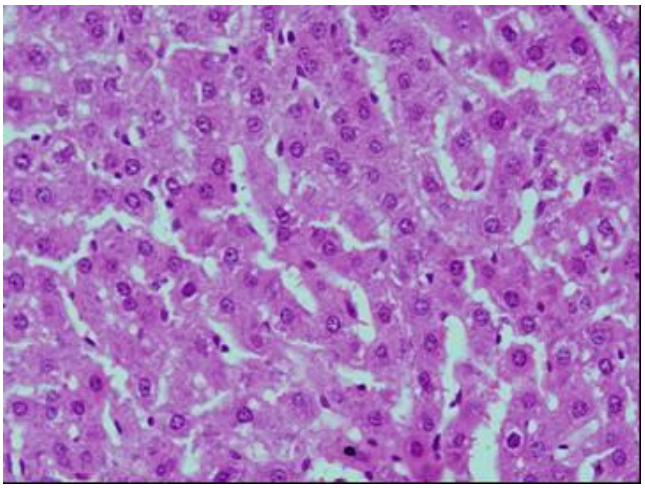
(Fig. 35). A photomicrograph in a liver section of rat treated with the extract alone showing normal hepatocytes structure.

(HX&E X 400)



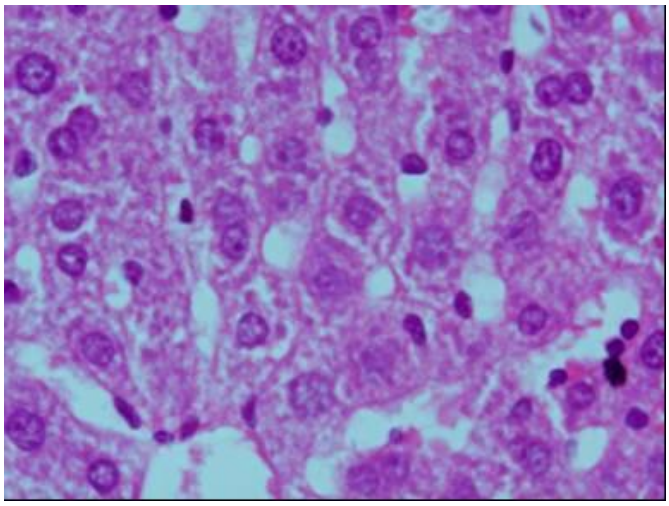
(Fig. 36). A photomicrograph in a liver section of rat treated with the extract alone showing normal hepatocytes and nuclei containing one or two nucleolus.

(HX&E X ١٠00)



(Fig. 37). A photomicrograph in a liver section of rat fed aflatoxin-contaminated diet and treated with the extract showing the regeneration of normal hepatocytes structure.

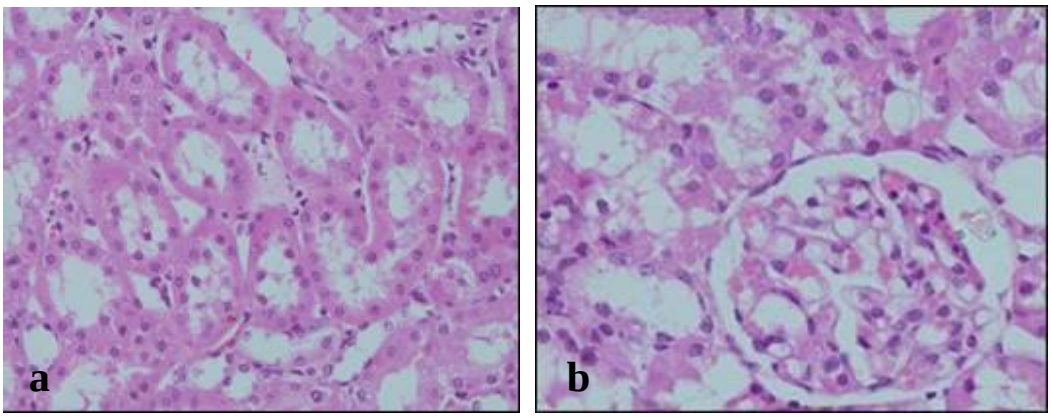
(HX&E X 400)



(Fig. 38): A photomicrograph in a liver section of rat fed aflatoxin-contaminated diet and treated with the extract showing normal hepatocytes, nuclei and central vein.

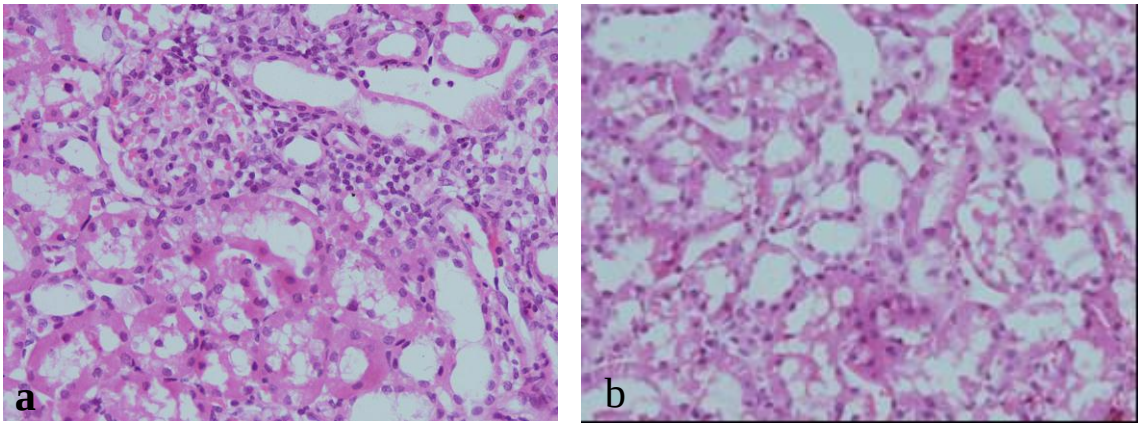
(HX&E X ١٠٠)

The histological study of the kidney revealed normal tubules and glomeruli in the kidney section of the control rats (Fig. 39a, b). The histological examination of the kidney sections of the group fed aflatoxin-contaminated diet showed massive intertubular infiltration (Fig. 40 a) and tubular epithelial cells vacuolar degeneration, cloudy swelling with pyknotic nuclei and tubular sclerosis (Fig 40b). The histological examination of the sections of kidneys in the rats fed aflatoxin-contaminated diet and treated with the extract showed moderate tubular improvement (Fig. 41a,b).



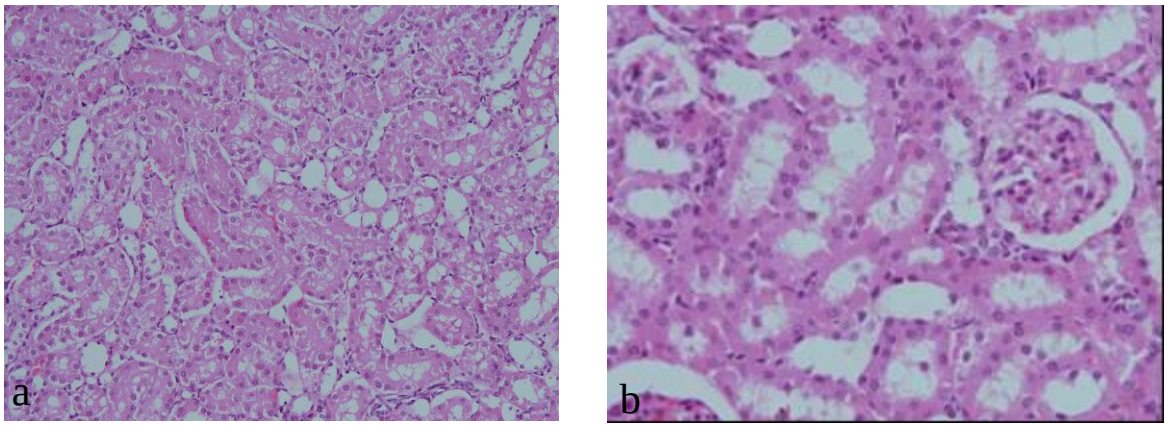
(Fig. 39). A photomicrograph in kidney section of control rat showing normal tubules and glomeruli.

(HX & E. X 200& 400)



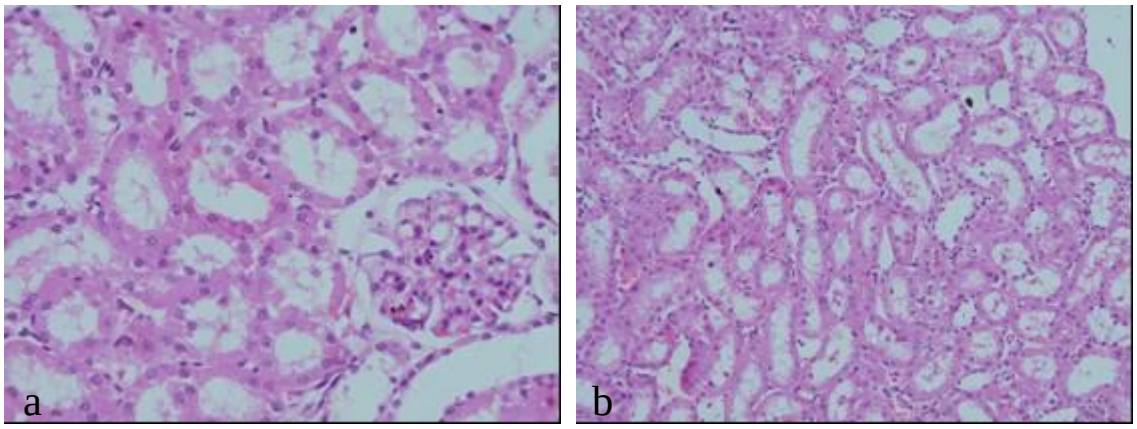
(Fig: 40) A section in kidney of rats fed aflatoxin-contaminated diet showing massive intertubular infiltration (a), and tubular epithelial cells vacuolar degeneration and cloudy swelling with pyknotic nuclei as well as tubular sclerosis (b).

(HX & E. X 200)



(Fig. 41): A field in a kidney section of rat treated with the extract alone showing tubular lumen obliteration and some epithelial cells degeneration (a), and normal structure tubules and glomeruli (b).

(HX & E. X 200& 400)



(Fig. 42). A section in kidney of animals fed aflatoxin-contaminated diet and treated with the extract showing moderate improvement in the tubular (a) and the glomeruli (b).

(HX & E. X 200 & 400)

The histological studies of the liver were further confirmed by the histochemical study of the DNA content in liver tissues stained by Feulgen stain. The results revealed that the liver sections in the control group showed normal Feulgen reaction and normal distribution of DNA (Fig. 42). The histochemical results of the liver in the animals fed aflatoxin-contaminated diet showed a

decrease in DNA content as demonstrated by the mild decrease in Feulgen reaction (Fig. 44). Animals treated with the extract alone showed normal distribution in DNA as demonstrated by the normal reaction of Feulgen stain (Fig. 45). Whereas the liver section in the rats fed aflatoxin-contaminated diet and treated with the extract showed significant improvement in DNA content as demonstrated by the marked reaction of Feulgen reaction (Fig. 46).

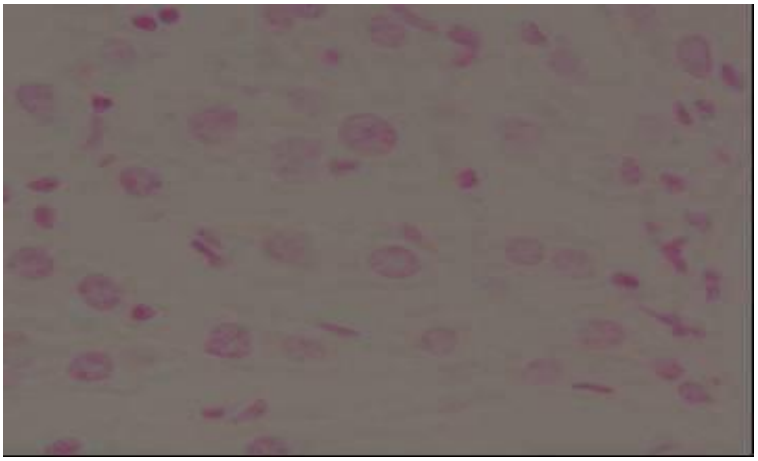


Fig. (43). Photomicrograph of liver section of control group showed normal reaction and normal distribution of DNA.

(Feulgen stain X1000)

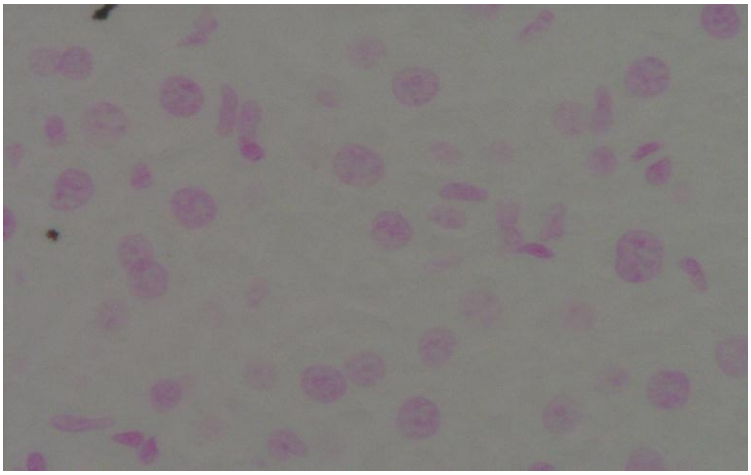


Fig. (44). Photomicrograph of liver section from rats fed aflatoxin-contaminated diet showed weak content of DNA and mild decrease in Feulgen reaction.

(Feulgen stain X1000)

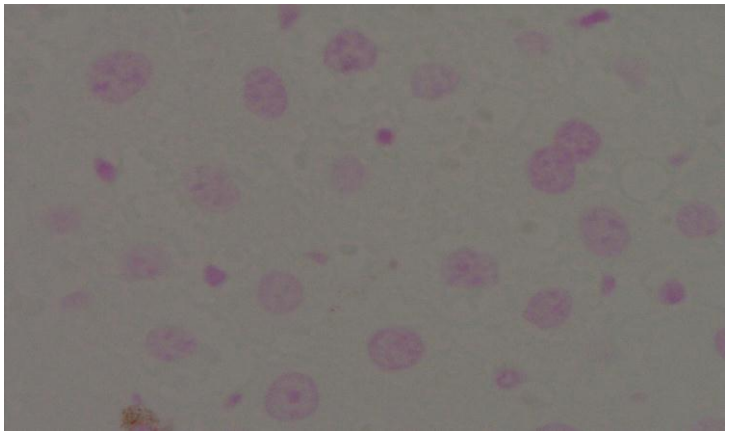


Fig. (45). Photomicrograph of liver section from animals treated with the extract alone showed normal content of DNA and marked reaction of Feulgen reaction.

(Feulgen stain X1000)

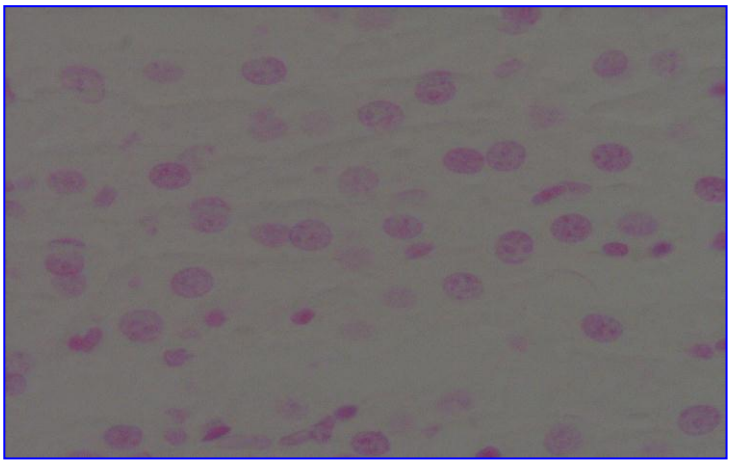


Fig. (46). Photomicrograph of liver from section different the animals fed aflatoxin-contaminated diet and treated with the extract showed marked increase in DNA content and marked reaction of Feulgen reaction.

(Feulgen stain X1000)

The histochemical results of the kidney sections of the different groups reveled that DNA content in the control kidney appeared normal as demonstrated by the normal reaction of the Feulgen reaction (Fig. 47). Animals fed aflatoxin-contaminated diet showed a decrease in DNA content in the renal tubules and glomeruli (Fig. 48). The kidney of the animals treated with the extract alone showed normal content of DNA in the renal tubules and glomeruli (Fig. 49). On the other hand, animals fed aflatoxin-contaminated diet and treated with the extract showed a significant improvement in the DNA in the tubules and glomeruli (Fig. 50).

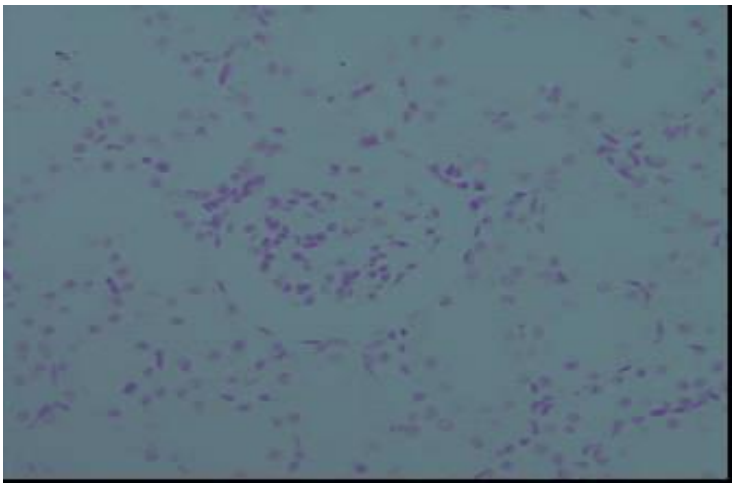


Fig. (47). Photomicrograph of kidney section from control group showing normal DNA distribution and normal reaction of Feulgen

(Feulgen stain X 200)

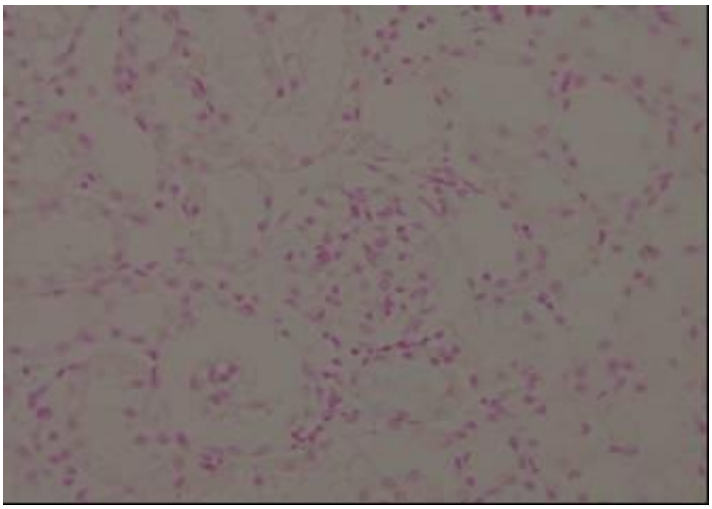


Fig. (48). Photomicrograph of kidney section from the group fed aflatoxin-contaminated diet showing mild DNA content in renal tubules and glomeruli.

(Feulgen stain X200)

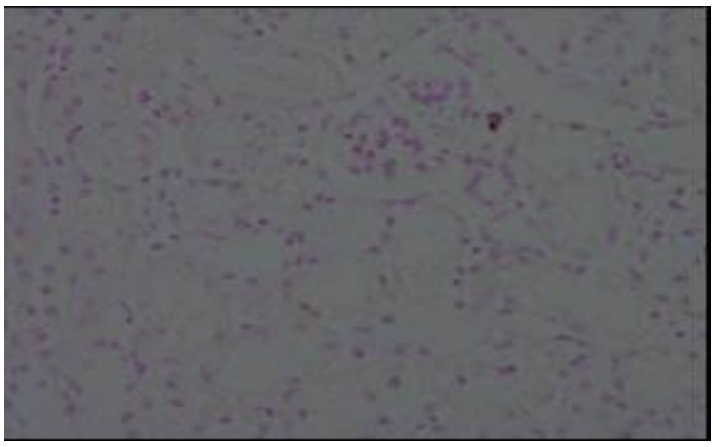


Fig. (49). Photomicrograph of kidney section from the animals treated with the extract alone showing normal DNA content.

(Feulgen stain X 200)

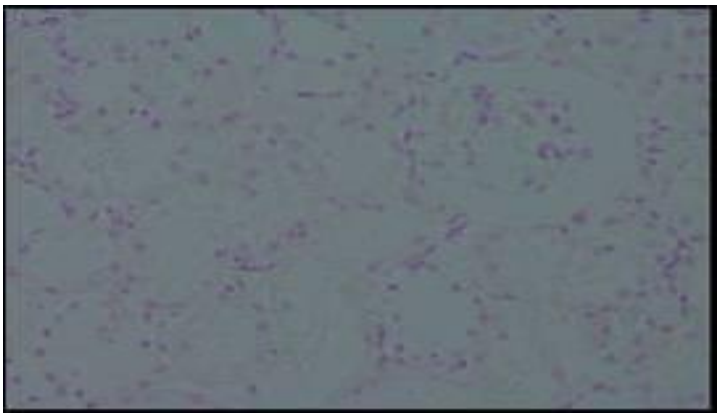


Fig. (50). Photomicrograph of kidney from the animals fed aflatoxin-contaminated diet and treated with the extract showing marked reaction and normal DNA content.

(Feulgen stain X200)