RESULTS AND DISCSSION

Aflatoxins are secondary mould metabolites known to be highly toxic and potential carcinogens. They have been detected in various food commodities from many parts of the world and are presently considered as one of the most dangerous contaminants of food and feed.

1. Chemical survey of aflatoxins in raw and roasted peanut

Aflatoxins are common contaminants of peanut and peanut-based products. Survey for aflatoxins in commercially available peanut was carried out in Great Cairo, Egypt. One hundred and four samples (52 raw peanut sample and other 52 roasted peanut samples) were purchased from markets of all over Cairo. The samples were analyzed for aflatoxins (B_1, B_2, G_1 and G_2) and quantified by high performance liquid chromatography (HPLC) as mentioned in the experimental part (materials and methods). The obtained results are shown in Tables (2 and 4).

Table (1) and Figure (3) represent the data of analyzed raw peanut samples collected from Great Cairo markets. From the Figure the variation of contamination between samples which are contaminated with aflatoxin B_1 is very high, from 17 contaminated samples only one sample over three hundred μg/kg, two samples near to one hundred μg/kg, tow samples over 50 but less 100 and the rest of contaminated samples are below 50 μg/kg. That is may be due to the different cultivated area of peanut and of course the contamination differentiates

from area to area because of differentiation of weather and conditions of planted area. Data in the priviuos Table and Figure represent that 14 samples of raw peanut were contaminated aflatoxin B2. Only one sample was contain over 50 μg/kg, one sample over 20 μg/kg and the rest of contaminated samples (12 samples) ranged from 0.5 to less than 10 μg/kg.

Table 1. Monitoring of aflatoxins in 52 raw peanut samples collected from Great Cairo markets.

No. of contaminated samples	Aflatoxin B_1 (μg/kg)	Aflatoxin B_2 (μg/kg)
1	99.28	21.82
2	9.05	1.03
3	28.39	3.96
4	2.04	0.5
5	1.78	0.52
6	54.3	8.1
7	97.45	7.86
8	80.12	8.09
9	35.49	3.92
10	3.09	0.56
11	0.55	0
12	349.37	52.6
13	18.31	2.14
14	1.68	0
15	1.88	0
16	18.13	1.56
17	6.7	1.14

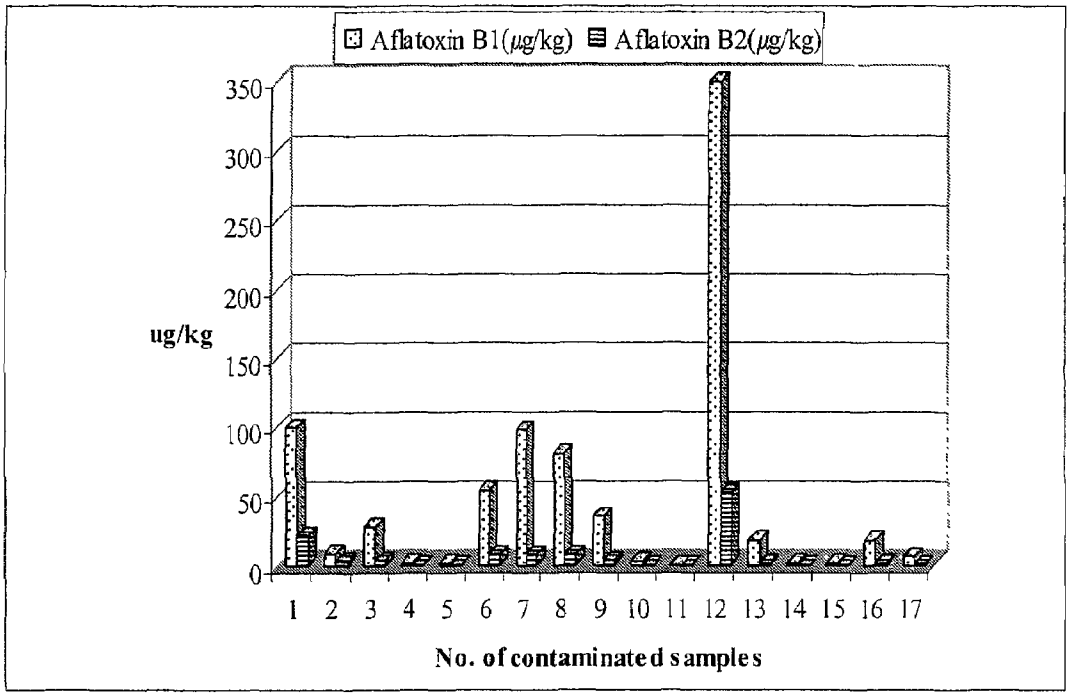

Fig. 3. Monitoring of aflatoxins in 52 raw peanut samples collected from Great Cairo markets.

Data in Table (2) show the number of naturally contaminated samples (raw) and the mean, minimum, maximum, number of violated samples comparing with Maximum Residue Limits (MRL) for the four types of aflatoxins and total aflatoxins. Seventeen samples were naturally contaminated with aflatoxin B_1 and B_2 with means 47.5 and 6.9 μg/kg, respectively, while only 21% of the analyzed samples were violated with B_1. The minimum and maximum of B_1 were 0.55 and 349.4 μg/kg, respectively, while the minimum and maximum of aflatoxin B_2 were 0.50 and 52.6 μg/kg, respectively. No violation for aflatoxin B_2 because of there is no MRL. All the samples were free from any detectable amount of aflatoxin $G_1$and G_2 while the mean, minimum and maximum of total aflatoxins were 54.4, 1.05 and 402 μg/kg, respectively. These data clear that the most mycotoxin found in raw peanut was aflatoxin B_1. The results of this study are almost agreed with the results of Chiou and Tsao (1997), Abdulkadar *et al.* (2000),

Itoh *et al.* (2001a, b), Abdulkadar *et al.* (2002), Caldas *et al.* (2002), Batatinha *et al.* (2003), Vaamonde *et al.* (2003), Younis and Malik (2003), Dharmaputra *et al.* (2004), Gurses and Erdogan (2004), Hifnawy *et al.* (2004), Mphande *et al.* (2004) and Razzazi-Fazeli *et al.* (2004), because aflatoxins contamination exist mainly due to conditions of temperature and humidity prevalent in the region of Marilia that are favourable for fungal growth.

Table 2. Number of naturally contaminated samples, mean, minimum, maximum, number of violated and percentages of violation in 52 analyzed raw peanut samples.

Type of toxin	No. of contaminated samples	Mean (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)	MRL (μg/kg)	No. of violated samples	Violation%
Aflatoxin B_1	17	47.5	0.55	349.4	5	11	21
Aflatoxin B_2	14	6.9	0.50	52.6	-	-	-
Aflatoxin G_1	0	-	-	-	-	-	-
Aflatoxin G_2	0	-	-	-	-	-	-
Total Aflatoxins	17	54.4	1.05	402.0	10	10	19

LOQ= Limit of quantitation for B_1 & B_2 = 0.5 μg/kg, for G_1 & G_2 = 1 μg/kg; MRL= Maximum Residue Limit as seated in Egyptian regulation; - = Less than Limit of Quantitation (LOQ)

Data in Table (3) and Figure (4) demonstrate that there is also differentiation among contaminated samples but in this case not only the different come from the differentiation of sources or type of peanut but also may due to the efficiency of roasting procedure. Roasting can decrease the amount of aflatoxin with range of 45-83% (Anonymous, 2003).

Table 3. Monitoring of aflatoxins in 52 roasted peanut samples collected from Great Cairo markets.

No. of contaminated samples	Aflatoxin B_1 (μg/kg)	Aflatoxin B_2 (μg/kg)
1	0.51	0
2	0.62	0
3	3.61	0.921
4	78.47	5.29
5	1.83	0

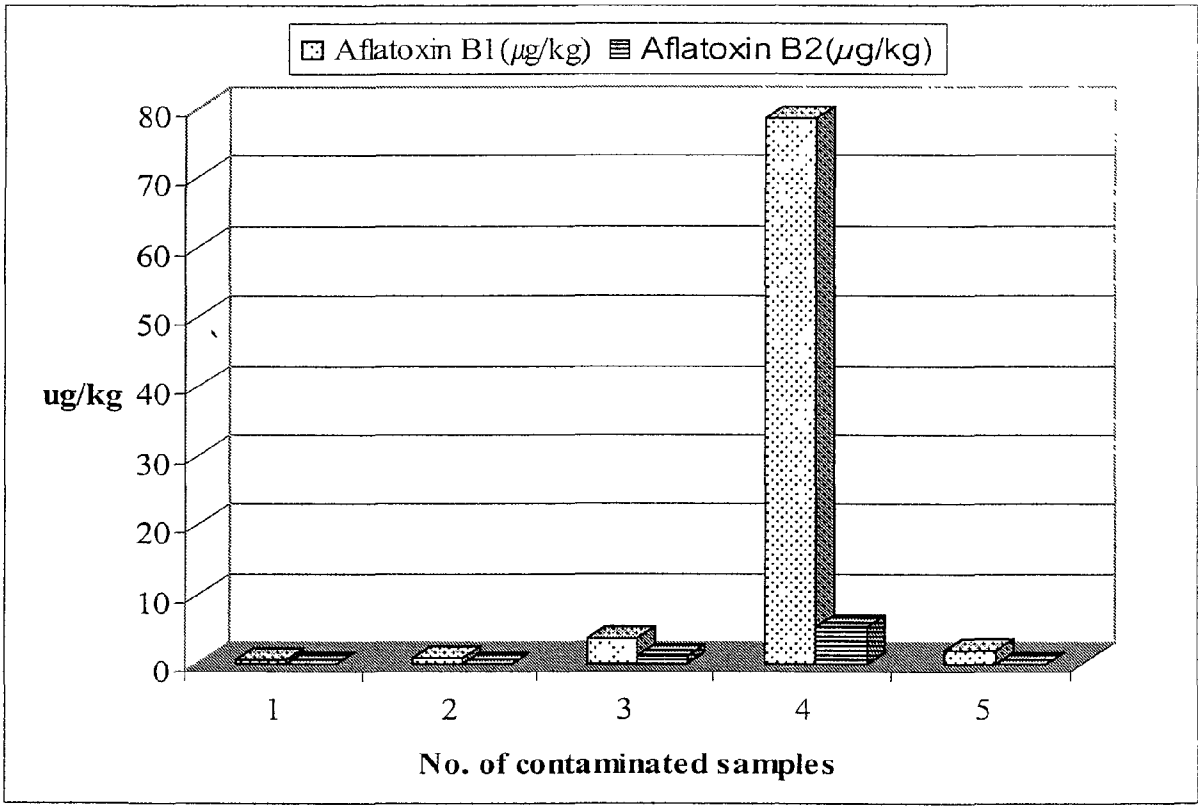

Fig. 4. Monitoring of aflatoxins in 52 roasted peanut samples collected from Great Cairo markets.

As in Table (4) roasted peanut samples were less than raw peanut in the content of aflatoxins. Five samples from 52 analyzed samples were naturally contaminated with aflatoxin B_1 and the mean, minimum and maximum amounts were 17.01, 0.51 and 78.47μg/kg,

respectively, while one sample was violated with aflatoxin B_1 with violation percentage 1.9%. Only two samples were contaminated with aflatoxin B_2 and mean, minimum and maximum amounts were 3.11, 0.92 and 5.29 μg/kg, respectively. Total aflatoxin was calculated by adding B1 with B2 for each sample. The mean, minimum and maximum amounts of total aflatoxins were 20.12, 1.43 and 83.76 μg/kg, respectively. The violation calculated on the MRL for total aflatoxins (10μg/kg) and only one sample was violated with percentage 1.9%. As in the raw peanut there was not any contamination with aflatoxin $G_1$and G_2. The results of this study are almost agreed with the results Fukal *et al.* (1987), Pluyer *et al.* (1987), Hag-Elamin *et al.* (1988), Singh *et al.* (1989), Njapau *et al.* (1998), Taha *et al.* (2001), Dawlatana *et al.,* (2002), Kumar-Harish *et al.*(2002), Thomas *et al.* (2003), Ogunsanwo *et al.* (2004) and Bankole *et al.* (2005). Because roasting conditions and initial aflatoxin concentration in raw peanuts determined the degree of mycotoxin reduction, with decreases ranging from 45 to 83%. Other studies using roasting demonstrated that aflatoxin concentrations could be decreased in nuts and oilseed meals and in corn (Conway *et al.,* 1978).

Tables (2 and 4) show that all samples of raw peanut and roasted peanut are free from any detected traces of aflatoxin G_1 and G_2. The results of this study are almost agreed with the results of Sabino (1989) and Shundo *et al.* (2003).

Table 4. Number of naturally contaminated, mean, minimum, maximum, number of violated and percentages of violation in 52 analyzed roasted peanut samples.

Type of toxin	No. of contaminated samples	Mean (μg/kg)	Minimum (μg/kg)	Maximum (μg/kg)	MRL (μg/kg)	No. of violated samples	Violation %
Aflatoxin B_1	5	17.01	0.51	78.47	5	1	1.9
Aflatoxin B_2	2	3.11	0.921	5.29	-	-	-
Aflatoxin G_1	0	-	-	-	-	-	-
Aflatoxin G_2	0	-	-	-	-	-	-
Total aflatoxins	5	20.12	1.43	83.76	10	1	1.9

LOQ= Limit of quantitation for B_1 & B_2 = 0.5 μg/kg; for G_1 & G_2 = 1 μg/kg; MRL= Maximum Residue Limit as seated in Egyptian regulation; -= Less than LOQ

2. Dietary intake and estimation daily intake (EDI) of aflatoxins B_1

Dietary exposure estimates set by scientific committees are based on threshold levels identified during toxicological studies, below which the toxins are considered not to cause adverse effects. A Tolerable Daily Intake (TDI) represents an estimate of the amount of a contaminant, expressed on a body weight basis, which can be ingested daily over a life time without appreciable health risks.

Dietary exposure to mycotoxins can be estimated for an average consumer and for high level consumers using data derived from National and Nutrition Surveys. These estimates can be compared with

Tolerable Daily or Provisional Tolerable Weekly Intake (TDI or PTWI) established by expert committees.

Estimation daily intake (EDI) is reflecting the situation of dietary intake and it was calculated by using the following equation to get the real number of toxin by ng/kg body weight /day (Codex Alimentarius, 2006).

$$\mathbf{EDI} = \frac{\textbf{food consumption of the product} \times \textbf{mean of contamination}}{\textbf{60 kg (average of body weight)}}$$

Data in Table (5) show the mean concentrations of aflatoxin B_1, B_2, G_1, G2 and total aflatoxins, in each studied commodity. Food consumption data of Egypt (FAO, 2006) show only there is certain number for consuming peanut (nuts) which is 0.26 g /day/ person, and this number is include peanut and peanut products.

Table 5. Means of contaminants in contaminated commodities.

Type of commodity	The means of aflatoxin (μg/kg)				
	B_1	B_2	G_1	G_2	Total
Raw peanut	47.5	6.9	0	0	54.4
Roasted peanut	17.01	3.11	0	0	20.12

Data in Table (6) show the estimation daily intake of aflatoxin B_1, because of the provisional maximum daily intake (PMDI) seated by Consell Supérieur hygiéne Publique de France CSHPF (1999) for aflatoxin B_1 instead of acceptable daily intake (ADI). Because of there is no available data of ADI or PMDI for aflatoxins B_2, G_1, G_2 and total aflatoxins the EDI's of them were not calculated.

Table 6. Estimated daily intake (EDI) of aflatoxin B_1 adults consuming peanuts.

Commodity	Mean conc. µg/kg	Consumption (g/person day) [a]	Estimated Daily Intake (EDI) In (ng /kg body weight /day) [b] Calculated value from mean	PMDI [C] (Af B1)	Rate % [d] Calculated value from mean
Raw peanut	47.5	0.26	12.35	0.15	8233
Roasted peanut	17.01	0.26	4.42	0.15	2948

a= Food and Agriculture Organization (FAO) 2006; b = EDI (ng /kg bow. /day) Calculated for each toxin by multiplying estimated values (µg/kg) by consumption (g/person/day) and divided by weights of adult person in average 60 kg; C = PMDI Provisional Maximum Daily Intake seated by CSHPF, 1999; d = Rate percentages calculated by dividing EDI by PMDI and multiplied by 100.

European countries decreased the maximum residue limits for mycotoxins especially aflatoxins in peanut because of the high health risk caused by mycotoxins. Whereas Joint FAO/WHO Expert Committee on Food Additives JECFA did not establish any values of ADI of aflatoxins and also there are no values of no observed effected level (NOEL) and low observed effected level (LOEL) of aflatoxins. For calculation of dietary intake and estimation daily intake of aflatoxins we used value of PMDI seated by CSHPF (1999) for aflatoxin B_1. Data in Table (5) represent the intake amounts of aflatoxins and EDI aflatoxin B_1 due to consuming peanut. Only aflatoxin B_1, B_2 and total aflatoxins were found in studied peanut samples.

The EDI and the percentages rate values were calculated in raw peanut and roasted for aflatoxin B_1, the values were 12.35 and 4.42

(ng/kg body weight/day), respectively, and the percentages values were 8233 and 2948%, respectively. From these results it is appear that the Egyptian persons who are living in Great Cairo are exposure to about thousands percentages more than the maximum exposure limit seated by CSHPF (1999). However the consuming amounts are varied among persons due to their consuming habits.

3. Toxicological studies of aflatoxin in roasted peanut naturally contaminated with aflatoxins (B_1 and B_2).

a. Determination of toxicological parameters

The present study investigated the effects of different diets supplemented with raw and roasted peanut naturally contaminated or not with aflatoxins on total protein, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum. Data in Table (7) and Figure (5) illustrate that, aflatoxins significantly increased serum total protein level in both raw and roasted peanut groups. After two weeks, the increase in the concentration of total protein was small and still in the normal value, but serum total protein level was greatly increased from 5.94 g/dl and 5.39g/dl in raw peanut group to 7.43g/dl and 7.14g/dl in raw peanut naturally contaminated with aflatoxins group and from 5.35g/dl and 5.50g/dl in roasted peanut group to 7.22g/dl and 6.99g/dl in roasted peanut naturally contaminated with aflatoxins after four and six weeks, respectively. From these results one may suggest that aflatoxins contaminated diets are more effective than aflatoxins free diets on serum total protein. As similar as in serum total protein, there was no significant different in serum albumin between all groups after two weeks. After continuous feeding for four weeks on the different diets, the present data recorded in Table

(8) and Figure (6) show that the concentration of serum albumin was significantly increased in rats fed raw and roasted peanut naturally contaminated with aflatoxins after four weeks by 29.76% and 27.70% compared with that fed free aflatoxins raw and roasted peanut, respectively. While after six weeks serum albumin concentration was significantly increased by 23.08% and 23.89%, respectively. Statistical analysis by Pearson correlation show that, there is very good linear positive correlation between serum total protein and serum albumin (Pearson Correlation, 0.944, P<0.000) as show in Table (11). It means that the increase in serum albumin level may lead to the increase in serum total protein level. These findings are in a good agreement with data published by Pozzi *et al.* (2000).

Table 7. Serum total protein level of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Treatment	Total protein (g/dl)			
	Zero time*	Two weeks	Four weeks	Six weeks
Raw peanut (control)	5.59 ±0.13	5.42[a] ±0.19	5.94[b] ±0.23	5.39[b] ±0.19
Roasted peanut		5.76[a] ±0.40	5.35[b] ±0.14	5.50[b] ±0.03
Raw peanut naturally contaminated with aflatoxins		6.48[a] ±0.42	7.43[a] ±0.20	7.14[a] ±0.30
Roasted peanut naturally contaminated with aflatoxins		5.82[a] ±0.17	7.22[a] ±0.42	6.99[a] ±0.21

*= Before any treatment; Each value represents the mean ± Standard Error; The mean values with different letters within a column indicate significant differences ($P< 0.05$).

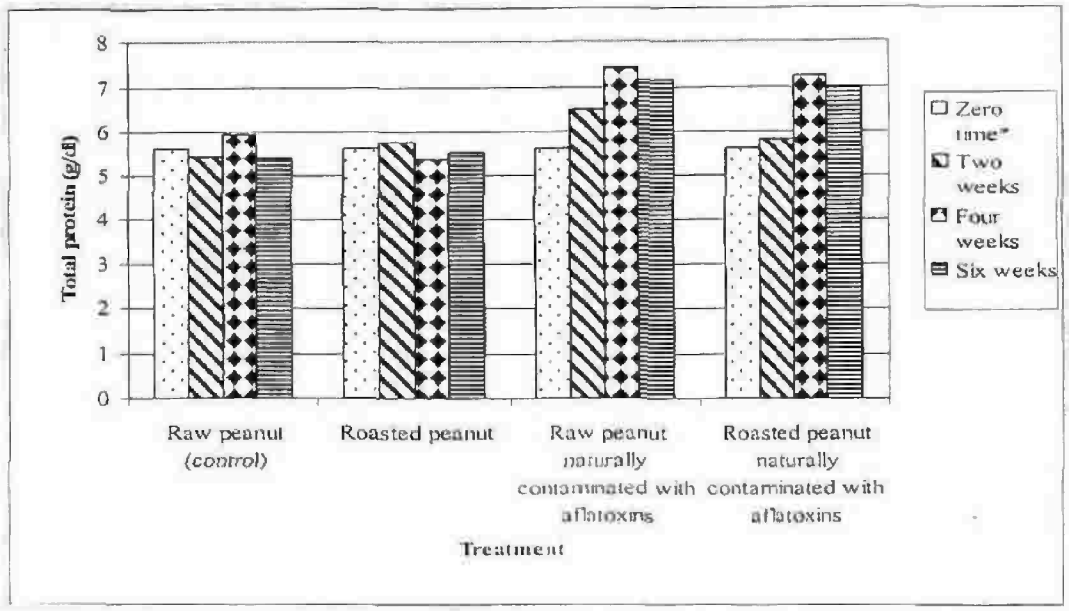

Fig. 5. Serum total protein level of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Table 8. Serum albumin level of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Treatment	Albumin (g/dl)			
	Zero time*	Two weeks	Four weeks	Six weeks
Raw peanut (control)	3.58 ±0.23	3.70[a] ±0.21	3.83[b] ±0.32	3.64[bc] ±0.29
Roasted peanut		3.86[a] ±0.27	3.61[b] ±0.24	3.39[c] ±0.29
Raw peanut naturally contaminated with aflatoxins		4.29[a] ±0.10	4.97[a] ±0.05	4.48[a] ±0.22
Roasted peanut naturally contaminated with aflatoxins		3.98[a] ±0.15	4.61[a] ±0.01	4.20[a b] ±0.07

*= Before any treatment; Each value represents the mean ± Standard Error; The mean values with different letters within a column indicate significant differences ($P< 0.05$).

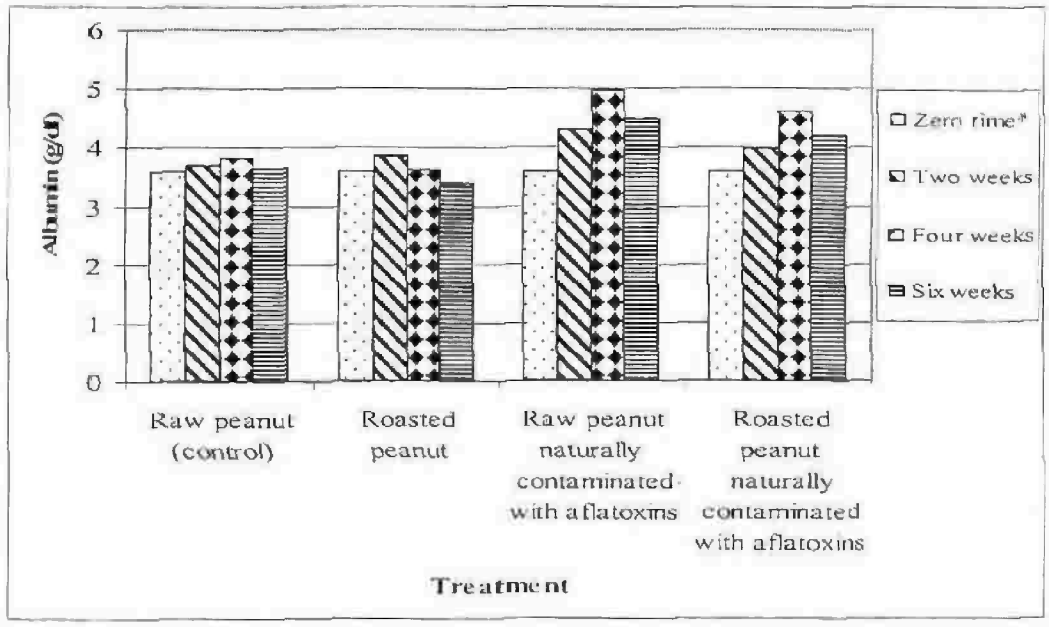

Fig. 6. Serum albumin level of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

The present data recorded in Table (9) and Figure (7) show also that, feeding normal rats on roasted peanut free aflatoxins diet directly increased serum alanine aminotransferase (ALT) activity of 2.01- and 2.03- times compared with that fed raw peanut free aflatoxins diet after four and six weeks, respectively. In case of raw peanut naturally contaminated with aflatoxins diet, ALT activity was increased by 2.33-, 1.95-, and 2.87- fold than that of the raw peanut free aflatoxins diet after two, four and six weeks, respectively. The activity of serum ALT in roasted peanut naturally contaminated with aflatoxins diets was also significantly increased by 2.4-, 3.1- and 3.38- fold than that of the raw peanut free aflatoxins diet, and by 2.27-, 1.54- and 1.67- fold than of the roasted peanut after two, four and six weeks, respectively. The highest value of ALT activity was recorded for rats fed diet

supplemented with roasted peanut naturally contaminated with aflatoxins after six weeks (88.00 U/L).

Table 9. Serum alanine aminotransferase (ALT) activity of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Treatment	ALT activity (U/L)			
	Zero time*	Two weeks	Four weeks	Six weeks
Raw peanut (control)	25.33 ±2.41	25.73 ^{b} ±2.15	23.20 ^{c} ±1.01	26.00 ^{d} ±2.00
Roasted peanut		27.33 ^{b} ±1.77	46.67 ^{b} ±3.53	52.67 ^{c} 5.21
Raw peanut naturally contaminated with aflatoxins		60.00 ^{a} ±3.06	45.33 ^{b} ±2.67	74.67 ^{b} ±1.33
Roasted peanut naturally contaminated with aflatoxins		62.00 ^{a} ±4.17	72.00^{a} ±6.12	88.00 ^{a} ±2.31

*= Before any treatment; Each value represents the mean ± Standard Error; The mean values with different letters within a column indicate significant differences ($P< 0.05$).

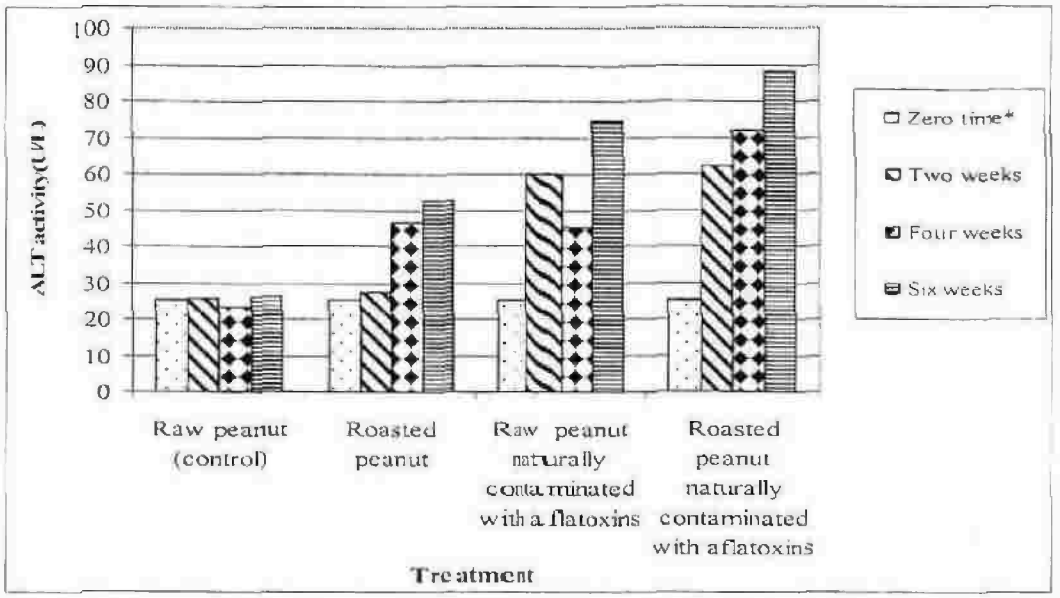

Fig. 7. Serum alanine aminotransferase (ALT) activity of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

The present data recorded in Table (10) and Figure (8) show that an increase in the activity of aspartate aminotransferase (AST) was also observed, but with only minor variation between treated groups. It must be noticed that the activity of AST in all groups was significantly increased compared with raw peanut free aflatoxin, group especially after four and six weeks. As similar as in ALT activity, the highest value of AST activity was recorded for rats fed diets supplemented with roasted peanut naturally contaminated with aflatoxins (38.68 U/L). In the present study, there is positive correlation between serum ALT and AST activities (Pearson Correlation=0.832, P<0.000). The activities of ATL and AST were increased in roasted peanut group and also aflatoxins contaminated peanut groups. It is indicated that not only aflatoxins, but also roasting treatment, is a rate limiting factor, for liver function. Such increase in ALT and AST activity can be attributed to

cell necrosis, changes in cell membrane permeability or impairment of biliary excretion (Pozzi *et al.*, 2000). It should be noted, however, that this effect was more marked in the groups treated with aflatoxins containing diets and affected especially ALT and AST, indicating an hepato-biliary dysfunction. As mention by Lynch *et al.* (1971) aflatoxins attack different cells in the body, especially liver cells. The activities of liver enzymes namely ALP, ALT and AST of animals given aflatoxins–contaminated diet were significantly increased in serum. This is likely due to liver damage and the release of these enzymes into the circulation. The present results are in a good agreement with data published by Gyamfi and Aniya (1998), Pozzi *et al.* (2000) and Kocabas *et al.* (2003).

Table 10. Serum aspartate aminotransferase (AST) activity of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Treatment	AST activity (U/L)			
	Zero time*	Two weeks	Four weeks	Six weeks
Raw peanut (control)	24.67 ±1.33	21.33 ^{c} ±0.67	24.33 ^{c} ±1.67	25.67 ^{b} ±2.06
Roasted peanut		20.00 ^{c} ±1.16	33.33 ^{ab} ±1.86	35.34 ^{a} ±1.20
Raw peanut naturally contaminated with aflatoxins		25.00 ^{b} ±0.58	28.33 ^{bc} ±0.33	36.31 ^{a} ±0.33
Roasted peanut naturally contaminated with aflatoxins		29.00 ^{a} ±0.58	38.00 ^{a} ±2.00	38.68 ^{a} ±1.77

*= Before any treatment; Each value represents the mean ± Standard Error; The mean values with different letters within a column indicate significant differences ($P< 0.05$).

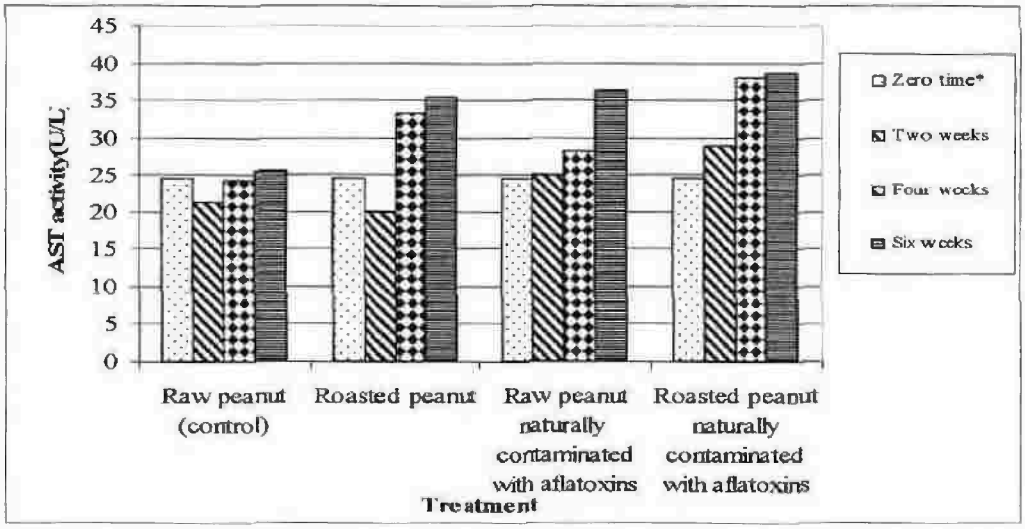

Fig. 8. Serum aspartate aminotransferase (AST) activity of rats fed diets supplemented with raw peanut, roasted peanut, raw peanut naturally contaminated with aflatoxins and roasted peanut naturally contaminated with aflatoxins for six weeks.

Table 11. Correlations between total protein, albumin, ALT and AST activities, in serum.

		Total protein	Albumin	ALT	AST
Total protein	**Pearson Correlation**	1.000	.944**	.645*	.469
	Sig. (1-tailed)		.000	.012	.062
	N	12	12	12	12
Albumin	**Pearson Correlation**	.944**	1.000	.480	.260
	Sig. (1-tailed)	.000		.057	.208
	N	12	12	12	12
ALT	**Pearson Correlation**	.645*	.480	1.000	.832**
	Sig. (1-tailed)	.012	.057		.000
	N	12	12	12	12
AST	**Pearson Correlation**	.469	.260	.832**	1.000
	Sig. (1-tailed)	.062	.208	.000	
	N	12	12	12	12

**Correlation is significant at the 0.01 level (1-tailed); *Correlation is significant at the 0.05 level (1-tailed).

b. Histopathological findings of treated rats liver

The combination between aflatoxin and roasted peanut comparing with non roasted and contaminated peanut was studied and the results were as follows in liver Figures (Figs. 9 - 31).

1. Group (1) of rats fed on raw free aflatoxin peanut

Sever congestion and dilation of the central veins associated with degenerative change in the surrounding hepatocytes was detected (Fig. 9 and 10). There were inflammatory cells infiltration, dilatation of the bile duct and congestion in the portal veins in the portal area (Fig. 11 and 12).

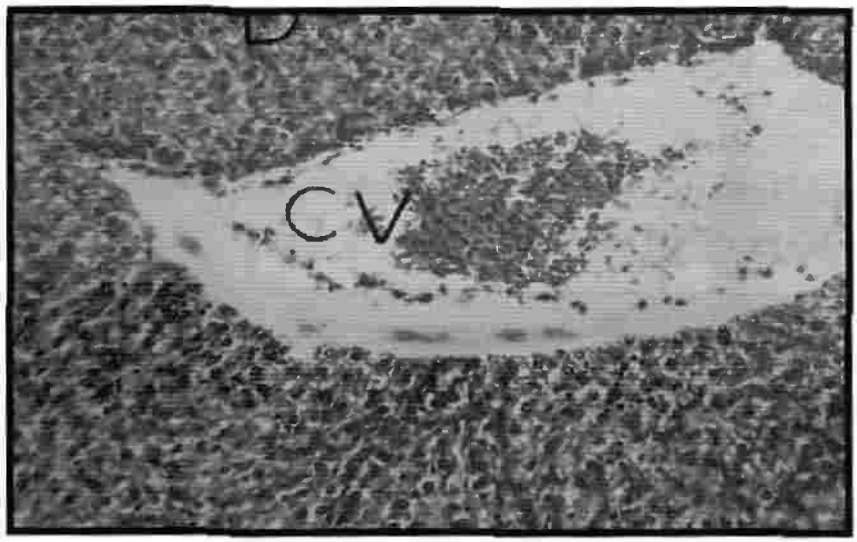

Fig. 9. Liver section of rats fed on raw free aflatoxin peanut (Gp. 1). Showing dilatation and congestion of central vein (cv) with degenerative change in the surrounding hepatocytes (D). (H&EX 40)

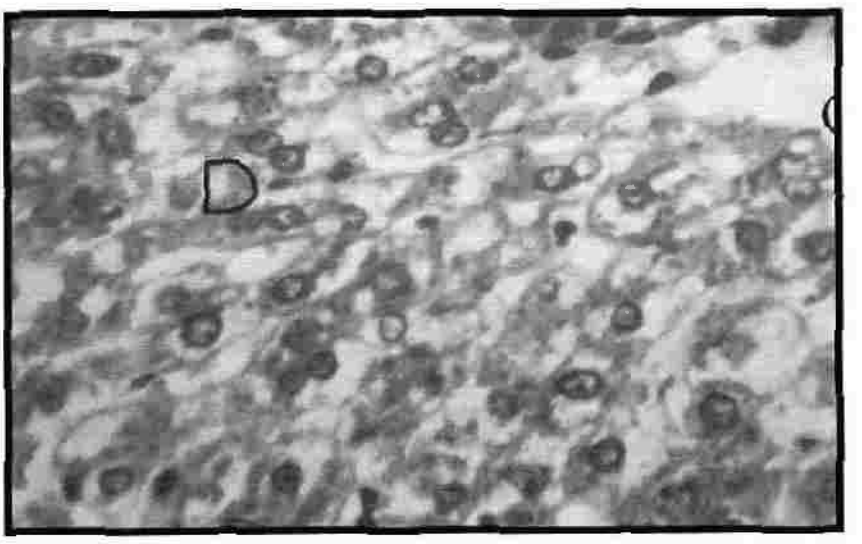

Fig. 10. Liver section of rats fed on raw free aflatoxin peanut (Gp. 1). Showing the magnification of (Fig.9) to identify the degenerated hepatocytes (D). (H&EX 160)

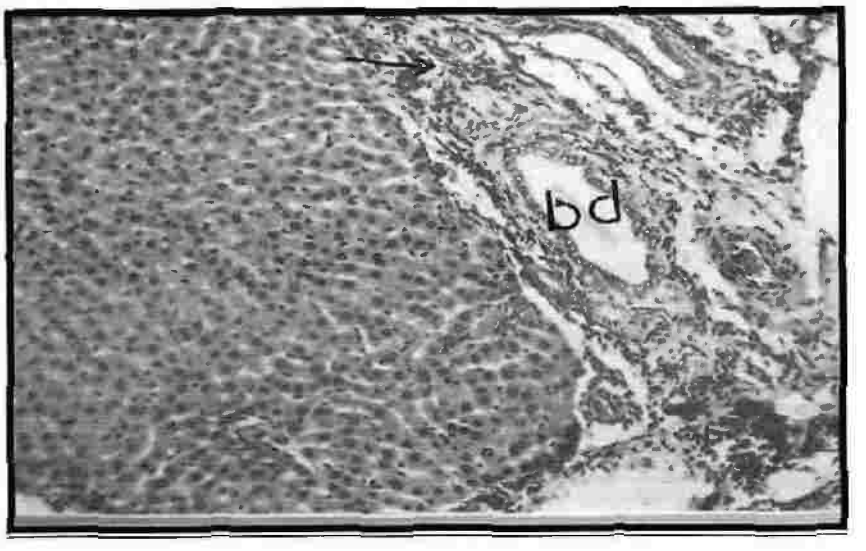

Fig. 11. Liver section of rats fed on raw free aflatoxin peanut (Gp. 1). Showing inflammatory cells infiltration (arrow) and dilated bile duct (bd) in the portal area. (H&EX 40)

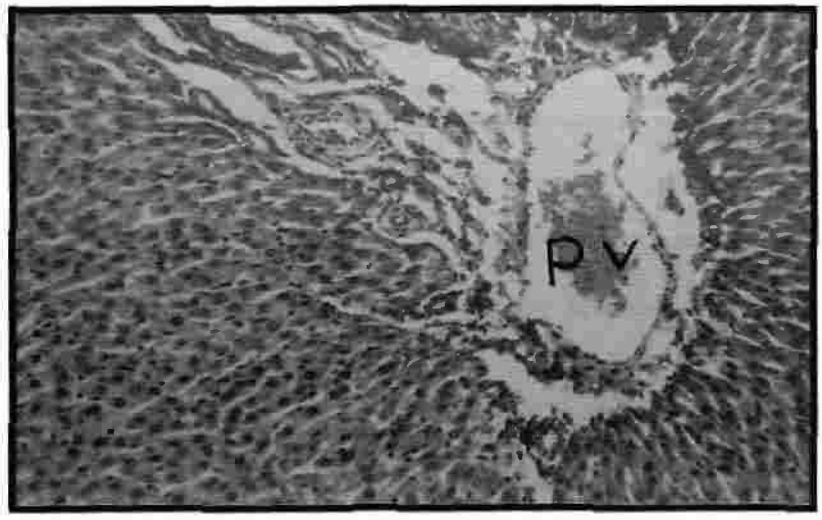

Fig. 12. Liver section of rats fed on raw free aflatoxin peanut (Gp. 1). Showing the dilatation and congestion of portal vein (pv). (H&E X 40).

2. Group (2) of rats fed on roasted free aflatoxin peanut

The hepatocytes showed different degenerations with fatty change (Fig. 13), in association with diffuse inflammatory cells infiltration as well as focal inflammatory cells aggregation with diffuse kupffer cells proliferation in between (Fig. 14 and 15) and dilatation with congestion in the central veins (Fig. 16). Sever dilatation of the portal vein with inflammatory cells infiltrations in the periductal tissue of the bile duct were observed in the portal area (Fig. 17 and 18).

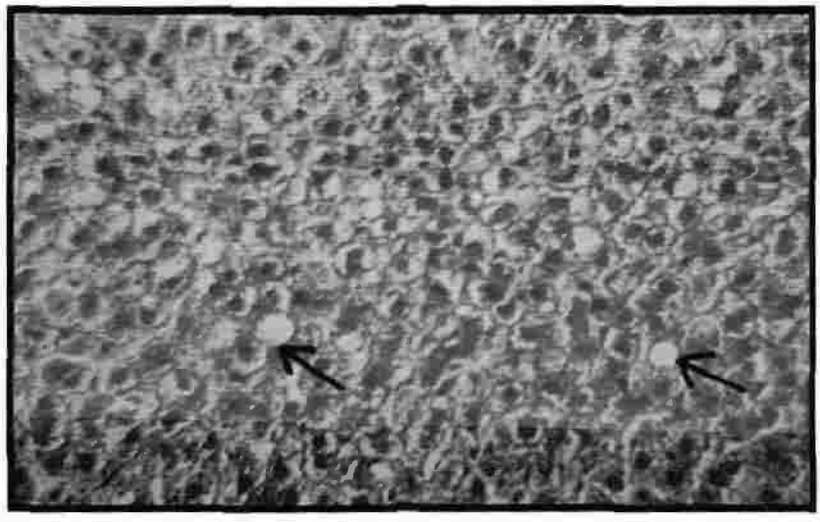

Fig. 13. Liver section of rats fed on roasted free aflatoxin peanut (Gp. 2). Showing fatty change in hepatocytes (arrow) with other degenerations. (H&E X 64)

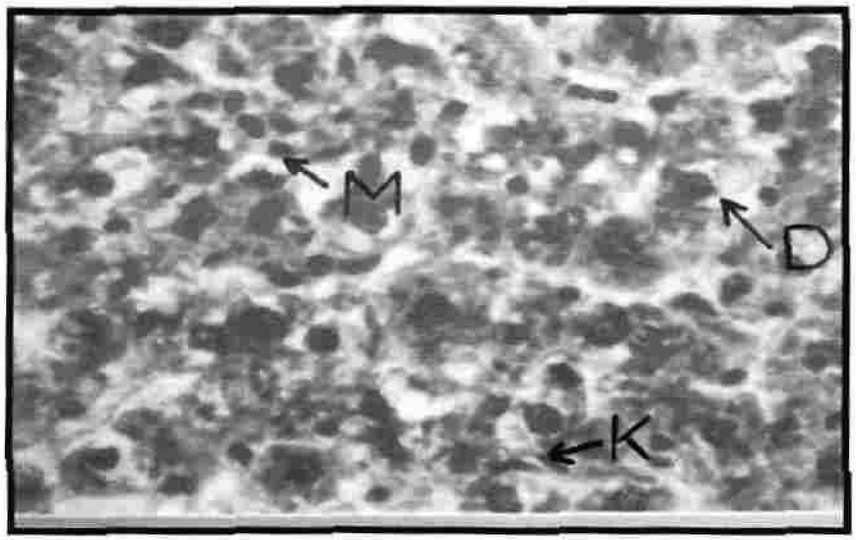

Fig. 14. Liver section of rats fed on roasted free aflatoxin peanut (Gp. 2). Showing mononuclear leucocytes inflammatory cells infiltration (arrow) with kupffer cells proliferation (arrow-k) in between the degenerated hepatocytes. (H&EX 160)

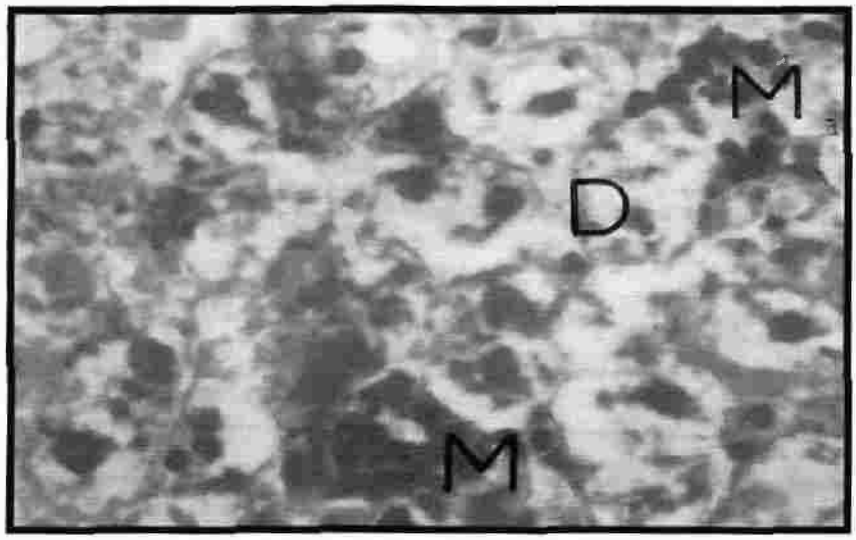

Fig. 15. Liver section of rats fed on roasted free aflatoxin peanut (Gp. 2). Showing focal mononuclear leucocytes inflammatory cells aggregations (M) in between the degenerated and necrosed (D) hepatocytes. (H&E X 160)

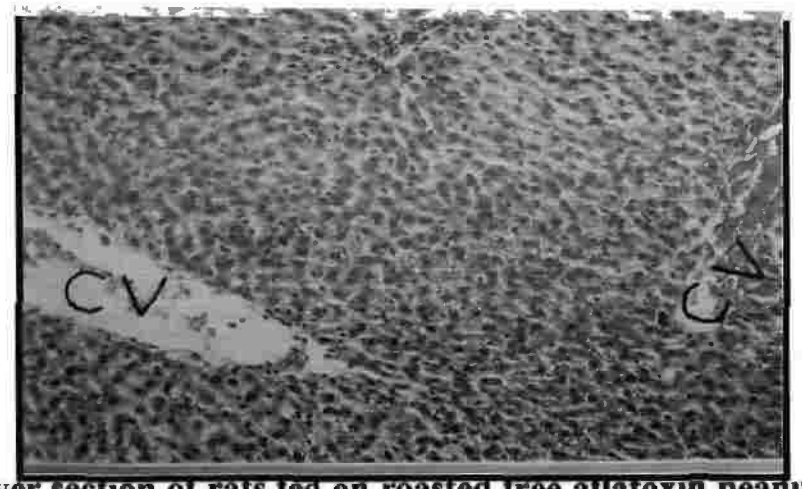

Fig. 16. Liver section of rats fed on roasted free aflatoxin peanut (Gp. 2). Showing sever congestion in the central vein (cv). (H&E X160)

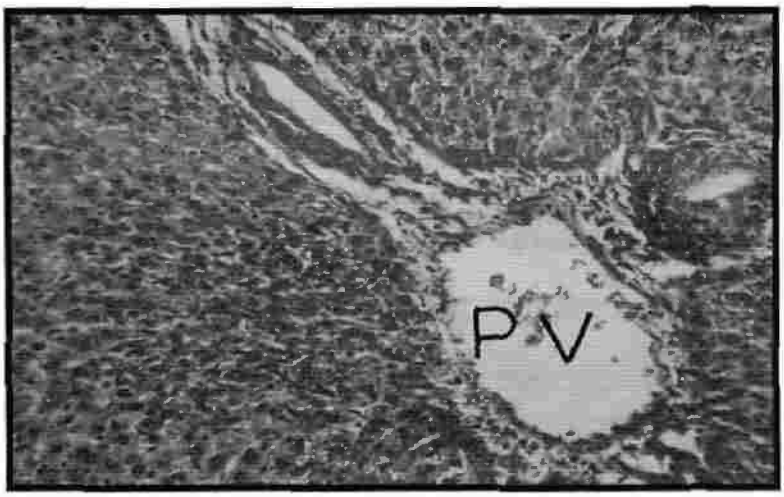

Fig. 17. Liver section of rats fed on roasted free aflatoxin peanut (Gp. 2). Showing severs dilatation of portal vein (pv). (H&E X 40)

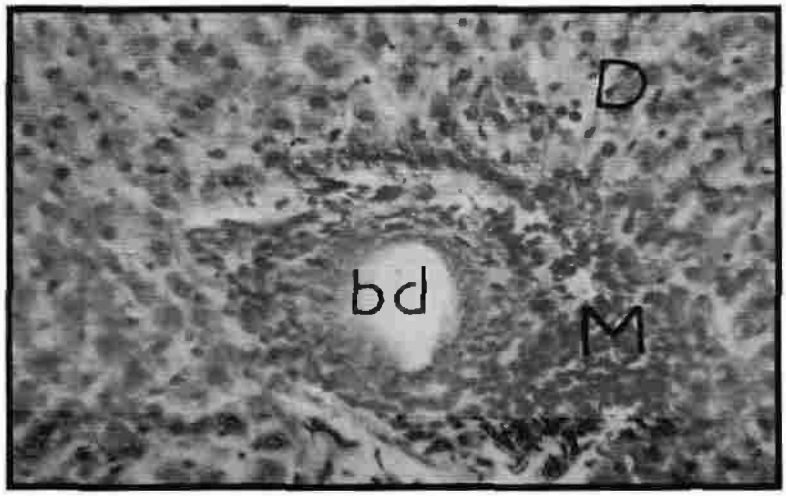

Fig. 18. Liver section of rats fed on roasted free aflatoxin peanut (Gp.2). Showing inflammatory cells infiltration in periductal tissue surrounding the bile duct (M). (H&E X64)

3. **Group (3) of rats fed on raw peanut naturally contaminated with aflatoxins**

Granular, vacuolar degenerations and fatty change were observed in the cytoplasm of the hepatocytes (Fig. 19). Fibrosis with inflammatory cells infiltration were originated from the portal area to the adjacent one to form a triangle tissue reaction (Fig. 20 and 21). There was severing dilatation and congestation in the central and portal veins (Fig. 22). The portal area showed periductal inflammatory cells infiltarion surrounding the bile duct (Fig. 23).

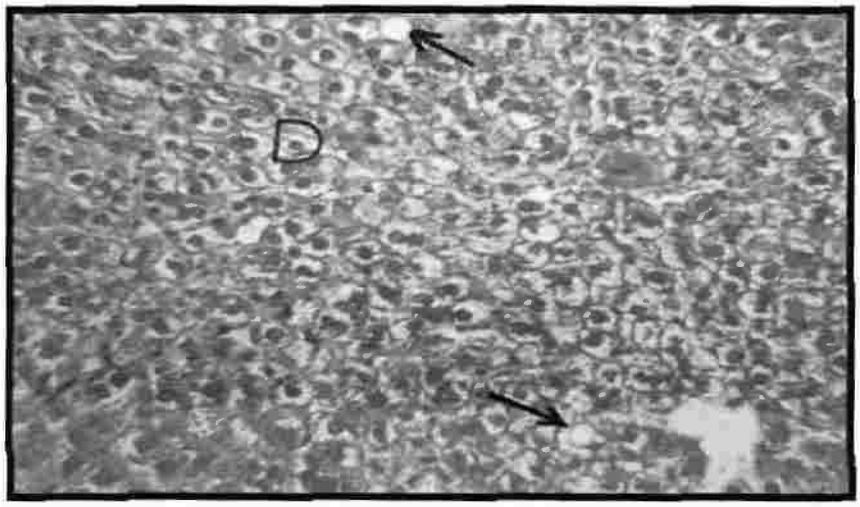

Fig. 19. Liver section of rats fed on raw peanut naturally contaminated with aflatoxins (Gp. 3). Showing different degenerative changes (granules degeneration, vacuolar degeneration and fatty change) in the hepatocytes (D) and (arrow). (H&E X 64)

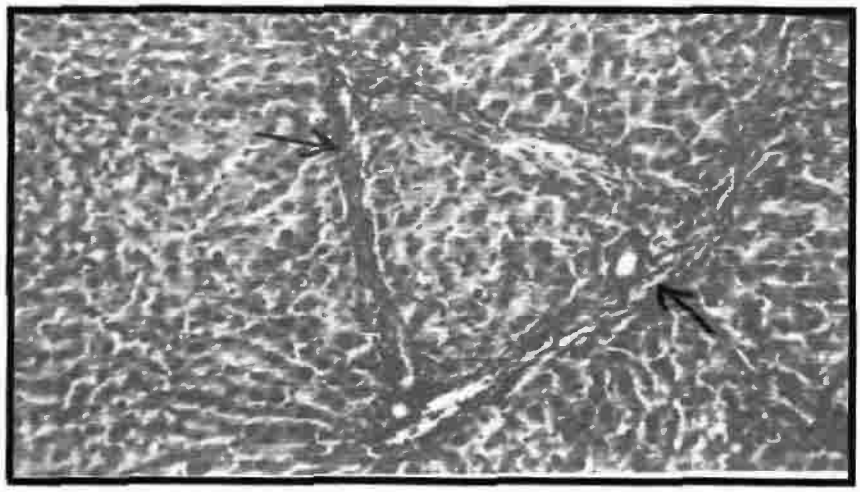

Fig. 20. Liver section of rats fed on raw peanut naturally contaminated with aflatoxins (Gp. 3).Showing inflammatory cells infiltration and fibrosis arising from the portal area to other making a triangle (arrow). (H&EX 40).

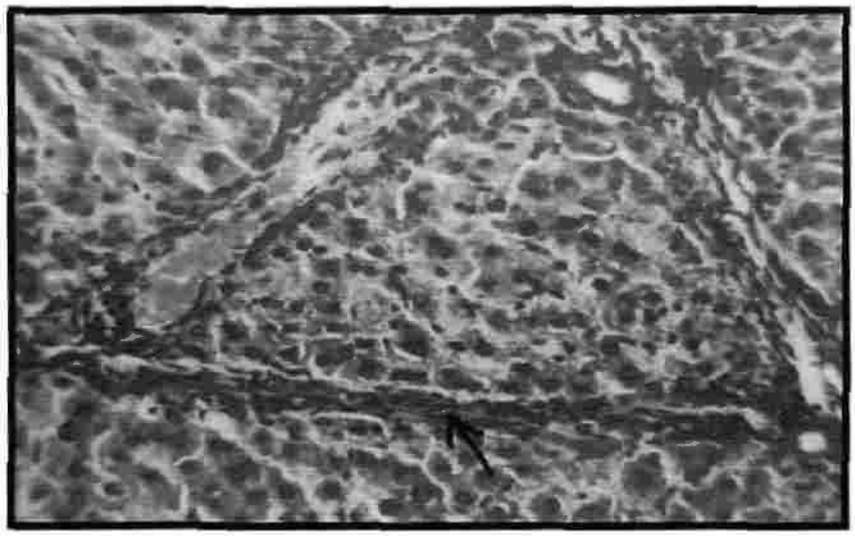

Fig. 21. Liver section of rats fed on raw peanut naturally contaminated with aflatoxins. (Gp. 3). Showing magnification of (Fig. 20) to identify the triangular inflammatory reaction (arrow) and diffuse kupffer cells proliferation in between the hepatocytes (k). (H&E X 64)

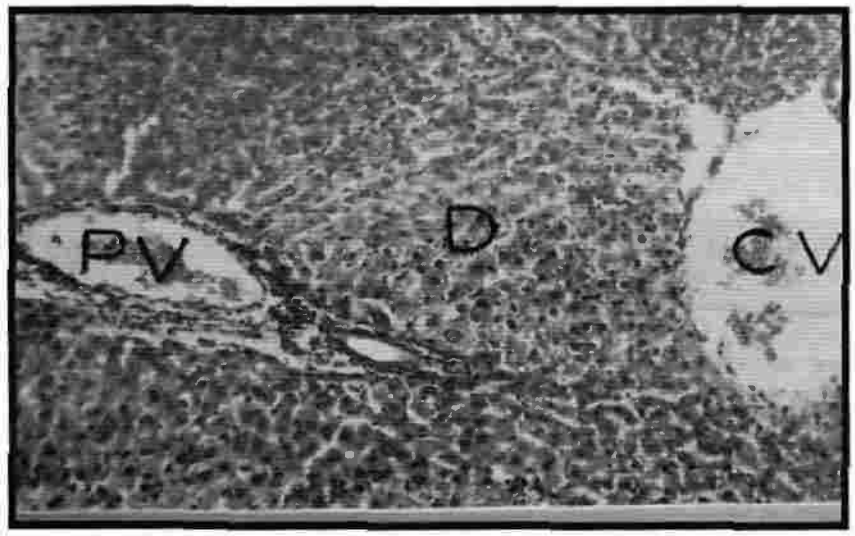

Fig. 22. Liver section of rats fed on raw peanut naturally contaminated with aflatoxins (Gp. 3). Showing severs dilatation and congestion in central veins (cv) and portal veins (pv). (H&E X 40)

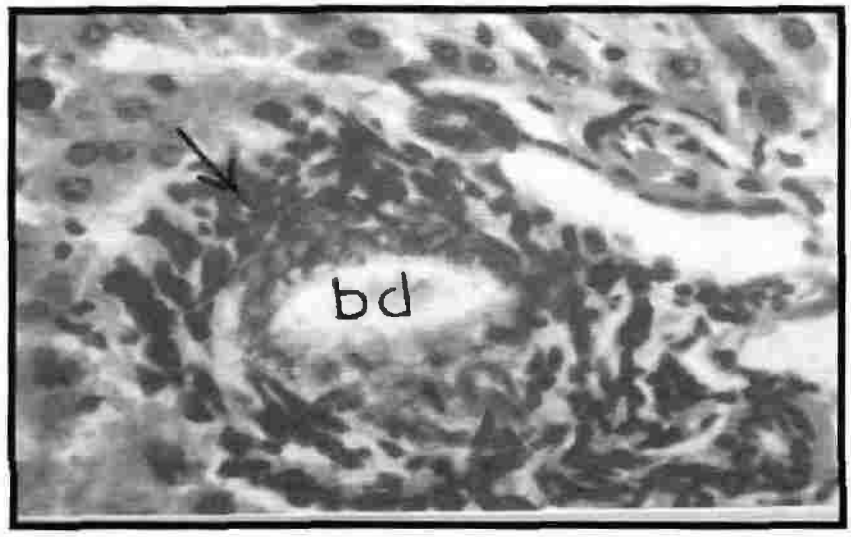

Fig. 23. Liver section of rats fed on raw peanut naturally contaminated with aflatoxins (Gp. 3). Showing inflammatory cells infiltration in the periductal tissue surrounding the bile duct (bd) in portal area. (H&E X 160)

4. Group (4) of rats fed on roasted peanut naturally contaminated with aflatoxins

Granular and vacuolar degeneration with fatty change were observed in the cytoplasm of the heptocytes (Fig. 24), associated with sever dilation and congestion in the central vein and focal inflammatory cells infiltration in the hepatic parenchyma Fig. 25). The portal area showed sever dilatation and congestion in the portal vein associated with periductal inflammatory cells infiltration and fibrosis surrounding the bile duct and allover the portal area (Fig. 26 and 27). Cytomegaly and karyomegaly were detected in cytoplasm and nuclei of the hepatocytes (Fig. 28), with appearance of multinucleated giant cell (Fig. 29). There was necrosis in some individual hepatocytes (Fig. 30), associated with dilatation and congestion in the hepatic sinusoids with extravasation of red blood cells in between the degenerated hypatocytes (Fig. 31).

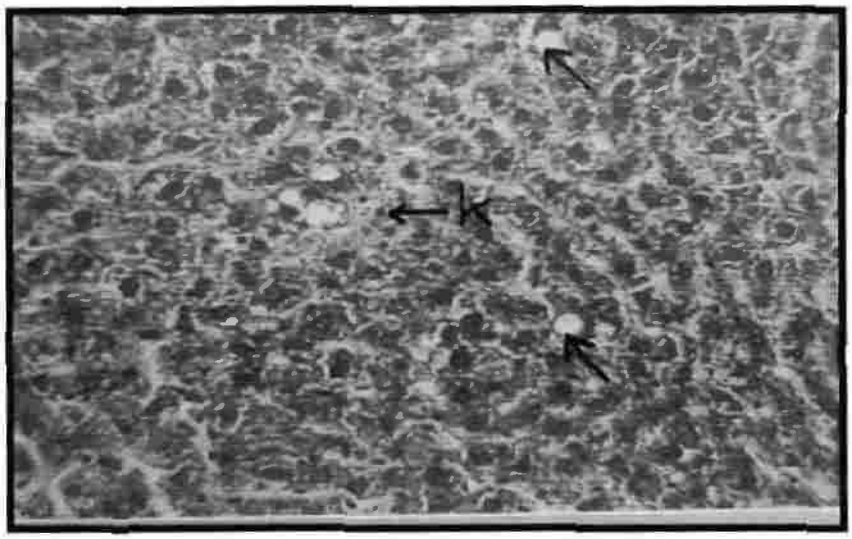

Fig. 24. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (Gp. 4). Showing granular and vacuolar degenerations and fatty changes in the hepatocytes (arrow) with diffuse kupffer cells proliferation in between (k-arrow). (H&E X 64)

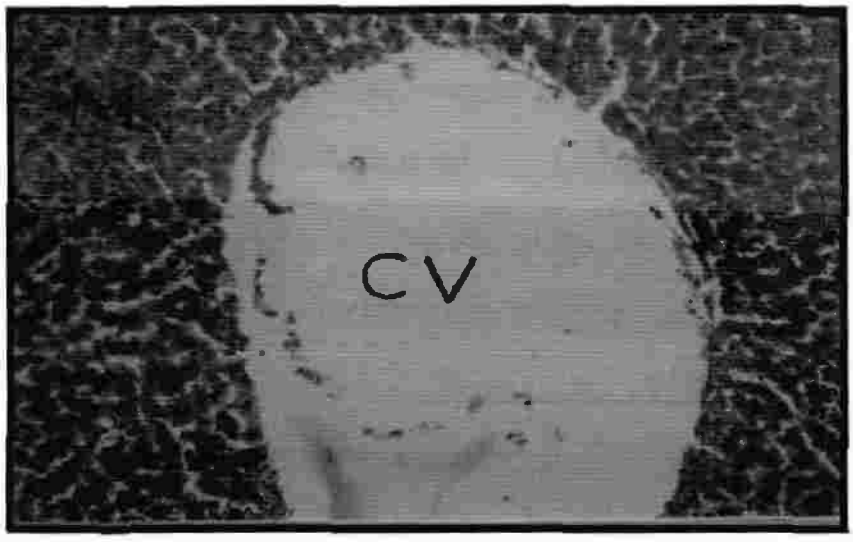

Fig. 25. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (gp. 4). Showing severs dilatation of central vein (cv) with focal inflammatory cells infiltration (M) in the hepatic parenchyma. (H&E X 40)

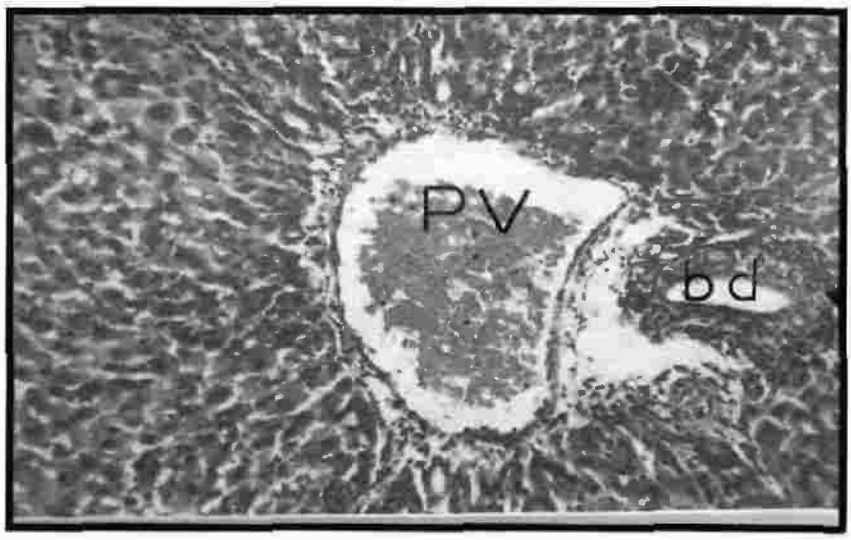

Fig. 26. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (Gp. 4). Showing severs dilatation of portal vein (pv) with periductal inflammatory cells infiltration and fibroblastic cells proliferation surrounding the bile duct (bd) in portal area. (H&E X 40)

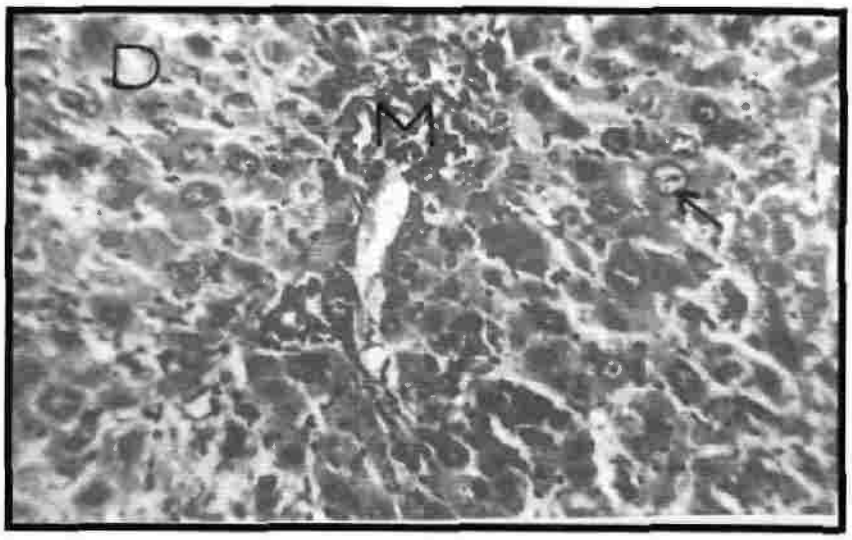

Fig. 27. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (Gp. 4). Showing mononuclear leucocytes inflammatory cells infiltration in the portal area. (H&E X 64)

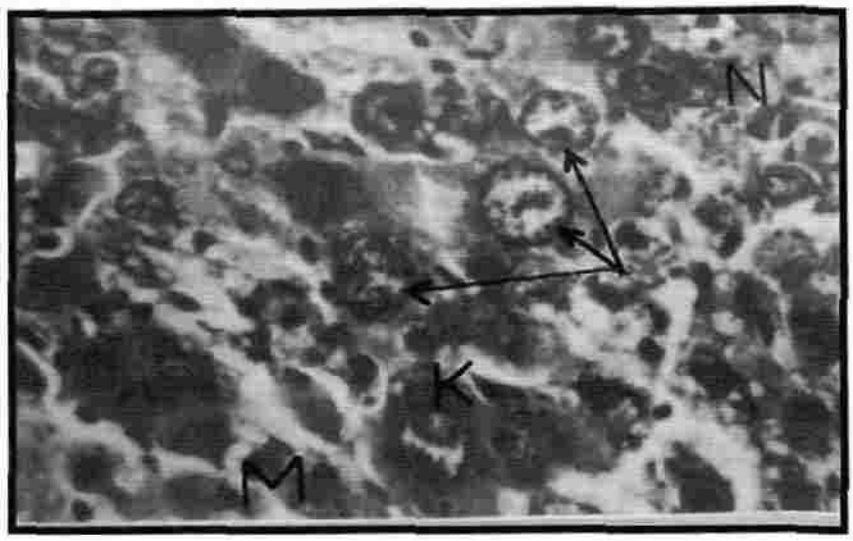

Fig. 28. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (Gp. 4). Showing cytomegaly and karyomegaly in the hepatocytes (arrows) with inflammatory cells infiltration (M) and kupffer cells proliferation (k) in between. (H&E X 160)

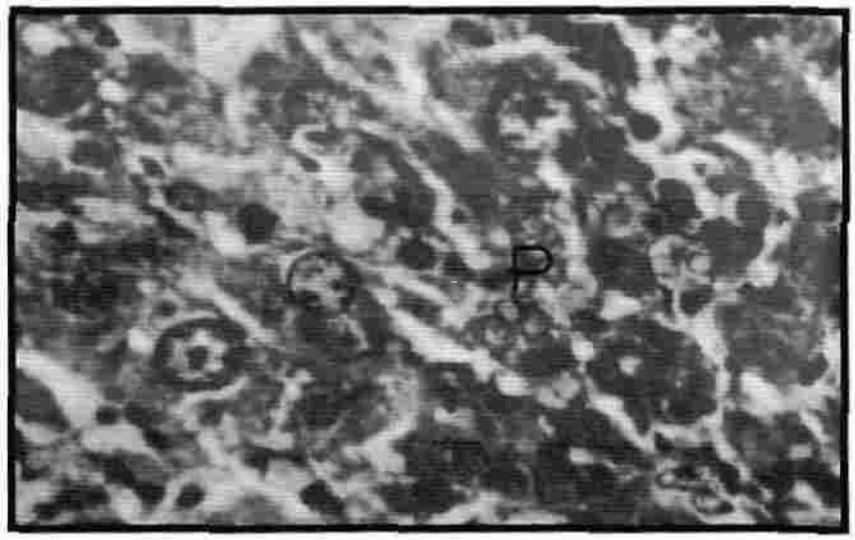

Fig. 29. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (Gp. 4). Showing multinucleated giant cells formation. (H&E X 160)

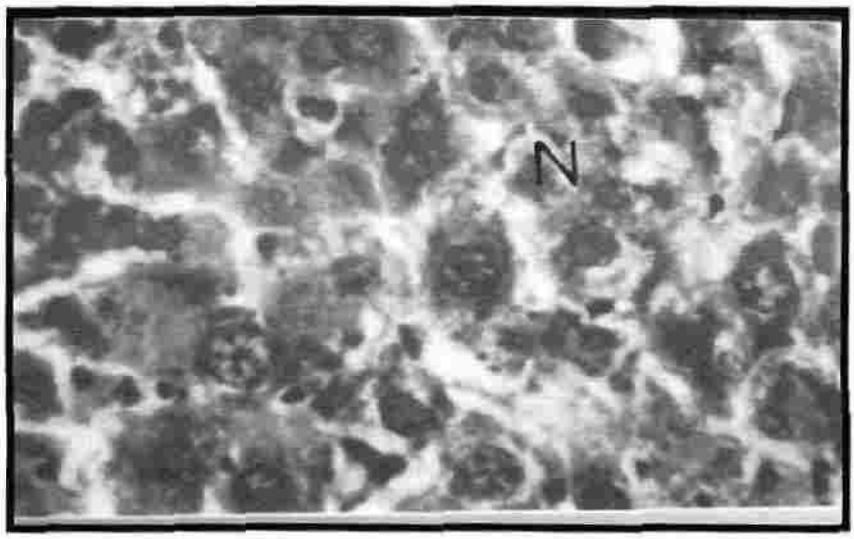

Fig. 30. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (gp. 4). Showing necrosis in individual hepatocytes (N). (H&EX 160)

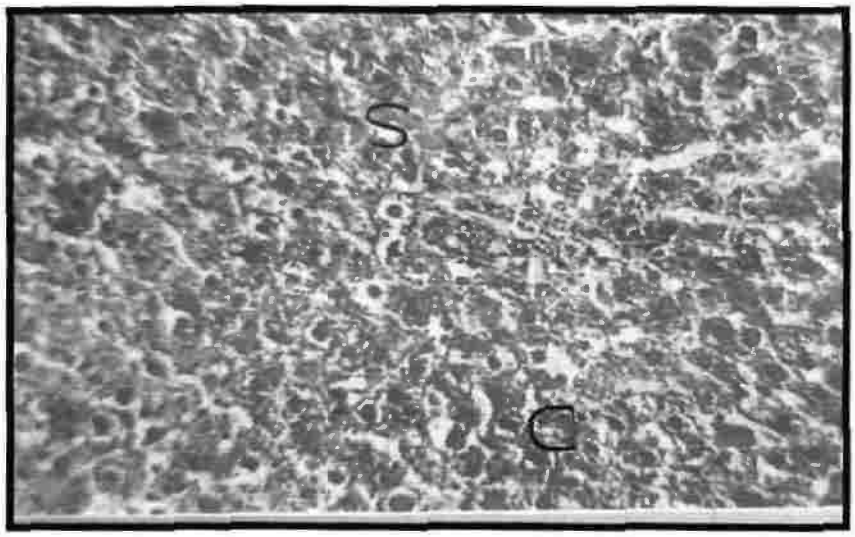

Fig. 31. Liver section of rats fed on roasted peanut naturally contaminated with aflatoxins (gp. 4). Showing dilatation and congestion with extravasated red blood Cells in the sinusoids in between the degenerated hepatocytes. (H&E X64)

Figures from 9 to 31 concluded that the combination between aflatoxin and roasted peanut for long duration administration is leading to dysplastic activity of the hepatocytes characterized by karyo and cytomegaly associated with the toxic syndrome characterized by degenerative changes and inflammatory reaction in diffuse manner

allover the hepatic tissue as well as in focal manner in the portal area the changes in the four groups are irreversible however changes in the other group are reversible. The toxicological effects of roasted peanut contaminated with aflatoxins are due to forming of free radical reactions which formed as results of increasing of enzymes activities, and increasing temperature. From the biochemical analysis and histopathological lesions it can be found that roasted peanut contaminated with aflatoxin is more effective on liver function than raw peanut. The results are matching with data of Temcharoen *et al.* (1978), Pozzi *et al.* (2000) and Karakilcik *et al.* (2004).

SUMMARY

This investigation was undertaken to survey the aflatoxins contamination of non roasted and roasted peanut in Cairo and evaluate thenutritionalvalueofnoncontaminatedand contaminated with aflatoxins non roasted and roasted peanut.

The obtained result could be summarized as follows:

One hundred and four samples of (raw peanut and roasted peanut) were monitored for determination of the four types of aflatoxins (B_1, B_2, G_1 and G_2).

Seventeen samples (raw peanut) were naturally contaminated with aflatoxin B_1 and B_2 with means 47.5 and 6.9 μg/kg, respectively, while only 21% of the analyzed samples were violated with B_1. The minimum and maximum of B_1 were 0.55 and 349.4 μg/kg, respectively, while the minimum and maximum of aflatoxin B_2 were 0.50 and 52.6 μg/kg, respectively. No violation for aflatoxin B_2 because of there is no MRL. All the samples were free from any detectable amount of aflatoxin $G_1$and G_2 while the mean, minimum and maximum of total aflatoxin were 54.4, 1.05 and 402 μg/kg, respectively. These data clear that the most mycotoxin found in raw peanut was aflatoxin B_1.

Roasted peanut samples were less than raw peanut in the content of aflatoxins. Five samples from 52 analyzed samples were naturally contaminated with aflatoxins B_1 and mean, minimum and maximum amounts were 17.01, 0.51 and 78.47μg/kg, respectively. While one sample was violated with aflatoxin B_1 with violation percentage 1.9%.

Only two samples were contaminated with aflatoxin B_2 and mean, minimum and maximum amounts were 3.11, 0.92 and 5.29μg/kg, respectively. As in the raw peanut there was not any contamination with aflatoxin $G_1$and G_2.

The EDI and the percentages rate values were calculated in raw peanut and roasted for aflatoxin B_1, and the percentages values were 8233 and 2948%, respectively. From these results it is appear that the Egyptian persons who are living in Great Cairo are exposure to about thousands percentages more than the maximum exposure limit seated by CSHPF (1999).

The present study investigated the effects of different diets supplemented with raw and roasted peanut naturally contaminated or not with aflatoxins on total protein, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum. Aflatoxins significantly increased serum total protein level in both raw and roasted peanut groups. After two weeks, the increase in the concentration of total protein was small and still in the normal value, but serum total protein level was greatly increased from 5.94 g/dl and 5.39g/dl in raw peanut group to 7.43g/dl and 7.14g/dl in raw peanut naturally contaminated with aflatoxins group and from 5.35g/dl and 5.50g/dl in roasted peanut group to 7.22g/dl and 6.99g/dl in roasted peanut naturally contaminated with aflatoxins after four and six weeks, respectively.As similar as in serum total protein, there was no significant different in serum albumin between all groups after two weeks. After continuous feeding for four weeks on the different diets, the present, the concentration of serum albumin was significantly

increased in rats fed raw and roasted peanut naturally contaminated with aflatoxins after four weeks by 29.76% and 27.70% compared with that fed free aflatoxins raw and roasted peanut, respectively.While after six weeks serum albumin concentration was significantly increased by 23.08% and 23.89.

ALT activity was increased by 2.33-, 1.95-, and 2.87- fold than that of the raw peanut free aflatoxins diet after two, four and six weeks, respectively .The activity of serum ALT in roasted peanut naturally contaminated with aflatoxins diets was also significantly increased by 2.4-, 3.1- and 3.38- fold than that of the raw peanut free aflatoxins diet, and of 2.27-, 1.54- and 1.67- fold than of the roasted peanut after two, four and six weeks, respectively. The highest value of ALT activity was recorded for rats fed diet supplemented with roasted peanut naturally contaminated with aflatoxins after six weeks (88.00 U/L).

An increase in the activity of aspartate aminotransferase (AST) was also observed, but with only minor variation between treated groups. It must be noticed that the activity of AST in all groups was significantly increased compared with raw peanut free aflatoxin, group especially after four and six weeks. As similar as in ALT activity, the highest value of AST activity was recorded for rats fed diets supplemented with roasted peanut naturally contaminated with aflatoxins (38.68 U/L).

The combination between aflatoxin and roasted peanut comparing with non roasted and contaminated peanut was studied and the results were as follows in liver.

Sever congestion and dilation of the central veins associated with degenerative change in the surrounding hepatocytes was detected .There were inflammatory cells infiltration, dilatation of the bile duct and congestion in the portal veins in the portal area.

The hepatocytes showed different degenerations with fatty change, in association with diffuse inflammatory cells infiltration as well as focal inflammatory cells aggregation with diffuse kupffer cells proliferation in between and dilatation with congestion in the central veins. Sever dilatation of the portal vein with inflammatory cells infiltration in the periductal tissue of the bile duct were observed in the portal area.

Granular, vacuolar degenerations and fatty change were observed in the cytoplasm of the hepatocytes. Fibrosis with inflammatory cells infiltration were originated from the portal area to the adjacent one to form a triangle tissue reaction. There was severing dilatation and congestation in the central and portal veins. The portal area showed periductal inflammatory cells infiltarion surrounding the bile duct.

Granular and vacuolar degeneration with fatty change were observed in the cytoplasm of the heptocytes, associated with sever dilation and congestion in the central vein and focal inflammatory cells infiltration in the hepatic parenchyma. The portal area showed sever dilatation and congestion in the portal vein associated with periductal inflammatory cells infiltration and fibrosis surrounding the bile duct and allover the portal area. Cytomegaly and karyomegaly were detected in cytoplasm and nuclei of the hepatocytes, with appearance of

multinucleated giant cell. There was necrosis in some individual hepatocytes, associated with dilatation and congestion in the hepatic sinusoids with extravasation of red blood cells in between the degenerated hypatocytes.

The combination between aflatoxin and roasted peanut for long duration administration is leading to dysplastic activity of the hepatocytes characterized by karyo and cytomegaly associated with the toxicsyndromecharacterized by degenerative changes and inflammatory reaction in diffuse manner allover the hepatic tissue as well as in focal manner in the portal area the changes in the four groups are irreversible however changes in the other group are reversible. The toxicological effects of roasted peanut contaminated with aflatoxins are due to forming of free radical reactions which formed as results of increasing of enzymes activities, and increasing temperature. From the biochemical analysis and histopathological lesions it can be found that roasted peanut contaminated with aflatoxin is more effective on liver function than raw peanut.