

INFLUENCE OF THE PROBIOTIC *PEDIOCOCCUS ACIDILACTICI* ON CHROMOSOMAL ABERRATIONS, CLINICOPATHOLOGICAL ALTERATIONS AND IMMUNOLOGICAL CHANGES IN AFLATOXICATED RABBITS

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SUMMARY

The present work was carried out to evaluate the effectiveness of the probiotic *Pediococcus acidilactici* in alleviating the toxicity of aflatoxin B₁ (AFB₁) in male New-Zealand white rabbits. Twenty four male New Zealand white rabbits, 800-1000 g body weight were used in the present work. Rabbits were randomly divided into four equal groups: Group (1) served as a control group. Group (2) was fed on crushed pellet diet mixed with the probiotic *Pediococcus acidilactici* at a dose of 100g/ton feed for six successive weeks. Group (3) was fed on crushed pellet diet artificially contaminated with 60 ug of AFB₁/kg of diet for six successive weeks while Group (4) was fed on crushed pellet diet mixed with the probiotic *Pediococcus acidilactici* (the same dose mentioned in G2) and artificially con-

taminated with 60 ug of AFB₁/kg of diet for six successive weeks. Evaluations were made for chromosomal aberrations, hemato-biochemical parameters, immunological changes as well as histopathological alterations. Our results showed that AFB₁ possesses a mutagenic effect. It significantly increases the frequency of chromosomal aberrations. AFB₁ induced significant decrease in the total leukocytic count associated with lymphopenia. Serum biochemical analysis revealed significant elevation in ALT, AST, GGT activities and BUN concentration with a marked decline in total proteins, albumin and globulins concentration. Significant decrease was recorded in the phagocytic percent and phagocytic index of neutrophils of aflatoxicated rabbits. Supplementation of the probiotic *Pediococcus acidilactici* to the aflatoxicated diet inhibited the mutagenic effect of AFB₁ as it sig-

nificantly decreased the frequency of chromosomal aberrations. Furthermore, *Pediococcus acidilactici* improved the hemato-biochemical alterations and nullified the phagocytic percent and phagocytic index of neutrophils of aflatoxicated rabbits.

Keywords: *Pediococcus acidilactici*, Aflatoxin B₁, chromosomal aberrations, hematobiochemical alterations.

INTRODUCTION

Mycotoxins are secondary metabolites of toxicogenic moulds that represent a great risk for human and animals. Aflatoxin B₁ (AFB₁) is among the most common mycotoxins produced by the genus *Aspergillus flavus* and *Aspergillus parasiticus* which are frequent contaminants of harvested feed and food stored under conditions of high humidity and temperature (Hassan et al. 2002). Their high toxicity to both animals and human makes AFB₁ the most dangerous known mycotoxins (Wilson and Payne, 1994). In addition to general toxicity, its biological effects include hepatocarcinogenic, immunosuppressive and mutagenic effects in different animal species (Sedmikova et al. 2001).The susceptibility of animals to aflatoxins is affected by species variations and nutritional factors (Pier, 1992). Rabbits are extremely sensitive to aflatoxin contaminated feed even at small concentrations (Newberne and Butler, 1969). Several methods have been tried in the

past to detoxify the feed ingredients from toxic metabolites. Previous studies suggested that the best approach to decontamination should be degradation by biological materials giving a possibility of aflatoxins removal under moderate conditions, without using harmful chemicals and without significant losses of the nutritive value and palatability of detoxified feed and feedstuffs (Bata and Lasztity, 1999). Nowadays, newer strategies for tackling the problem of mycotoxins have been developed.

Probiotics are live microbial feed supplement which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). It has been identified as a potential means to reduce availability of AFB₁ as well as other contaminants (Gratz et al. 2006).

The present work aimed to evaluate the effectiveness of the probiotic *Pediococcus acidilactici* in alleviating the toxicity of AFB₁ in male Newzealand white rabbits. We investigate the effect of *Pediococcus acidilactici* on chromosomal aberrations, clinicopathological alterations and immunological changes associated with aflatoxicated rabbits.

MATERIALS AND METHODS

Probiotic:

Pediococcus acidilactici (Bactocell) produced by lallemand, France was used as a probiotic. It was

used in a concentration of 100g/ton feed.

Aflatoxin B₁:

Aflatoxin B₁ was kindly obtained from Biochemistry Department, Animal Health Research, Institute, Dokki. It was mixed with the ration at a dose of 60 µg/kg crushed pellets.

Animals and experimental design:

Twenty four male New Zealand white rabbits, 800-1000 g body weight were used in the present work. Rabbits were kept in battery cages. Both feed and water were provided ad libitum. After an acclimation period of two weeks, rabbits were randomly divided into four equal groups:

Group (1): Rabbits in this group were fed on crushed pellet diet and served as a control group.

Group (2): Rabbits in this group were fed on crushed pellet diet mixed with the probiotic *Pedococcus acidilactici* at a dose of 100g/ton feed (Awaad et al. 2003) for six successive weeks.

Group (3): Rabbits in this group were fed on crushed pellet diet artificially contaminated with 60 µg of AFB₁/kg of diet (Karakilcik et al. 2004) for six successive weeks.

Group (4): Rabbits in this group were fed on crushed pellet diet mixed with the probiotic *Pedococcus acidilactici* (the same dose mentioned in G2) and artificially contaminated with 60 µg of AFB₁/kg of diet for six successive weeks.

Sampling:

Blood samples:

Three blood samples were collected from each group (5 rabbits/group) from the ear vein.

The first one was collected into clean dry tube containing EDTA (disodium salt of ethylenediamine tetra-acetic acid) every other week throughout the 6 weeks and used for hematological studies.

The second sample was collected into plain centrifuge tube every other week throughout the 6 weeks for serum preparation and used for biochemical studies.

The third one was collected on heparin anticoagulant solution (20 I.U. /ml blood) at the end of experimental period for measuring phagocytic activity of neutrophil.

Bone marrow samples:

They were collected at the end of experimental period from both femurs of the sacrificed rabbit (5rabbits/group) from each group for the cytogenetical analysis.

Histopathological samples:

Liver, kidneys, spleen and thymus samples were collected at the end of the experiment from all groups for histopathological studies.

Methods:

1- Chromosomal aberrations test:

Chromosomes from bone marrow cells were prepared according to the method of Tjio (1965).

The bone marrow was subjected to colchicine treatment (0.5 % solution, 0.1 ml culture), hypotonic treatment (KCL, 5.6 g/ L), fixed in acetomethanol, spread and stained by Giemsa stain diluted in 6.8 phosphate buffer. Fifty well spread metaphases were examined under the microscope for each rabbit to analyze the frequency and type of chromosomal aberrations.

2- Hematological studies:

The hematological studies included estimation of total erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and total and differential leukocytic count were performed according to Feldman et al. (2000).

3- Biochemical studies:

Serum was separated for determination of the following parameters: alanine (ALT) and aspartate (AST) amino transferase activities were performed according to the Reitman and Frankel (1957) method, gamma glutamyl transferase after Szasz, (1969). Blood urea nitrogen was determined by an enzymatic method after Patton and Grauch (1977) and serum creatinine according to Fabiny and Eringhausen (1971). Total serum proteins were assayed according to the biuret method after Weichselbaun (1964) and serum albumin after Dumas and Biggs (1972). Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA. Serum proteins fractions were separated and measured by electrophoresis using cellulose

acetate strips according to Keyser and Watkins (1972).

4- Phagocytic activity:

Phagocytic activity of neutrophils for cell mediated immune-response assessment using Dextran, 500.000 M.W. from Sigma according to the method described by Wilkinson (1981).

5- Histopathological studies:

Specimens from liver, kidneys, spleen and thymus were taken directly after sacrifice of the examined rabbits and fixed immediately in 10 % neutral buffered formalin solution. Paraffin sections of 4-6 μ thick were prepared and stained with hematoxylin and eosin (Bancroft et al. 1996).

6- Statistical analysis:

Data were compared across groups using analysis of variance (ANOVA). Data were expressed as mean \pm S.E. Level of significance of $P < 0.05$ was chosen to identify the significant differences (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Aflatoxin B₁ is a strong hepatotoxin affecting many organs and body systems as well as potent carcinogen (Eaton and Groopman, 1994). Interventions of aflatoxins focus either on improving crop quality and storage or on altering aflatoxins bioavailability. In the present study, trials were

made to evaluate the effectiveness of the probiotic *Pediococcus acidilactici* in reducing the harmful effects of AFB₁ in rabbits. Results of chromosomal aberrations of different experimental groups are shown in table (1). Cytogenetic analysis revealed that AFB₁ possesses a mutagenic effect as it significantly increases the frequency of both individual and total chromosomal aberrations of rabbits bone marrow cells (33.34 ± 1.33) in comparable to control group (8.33 ± 0.45). The observed chromosomal aberrations were categorized into structural and numerical aberrations. The observed structural aberrations were centromeric attenuation, deletion, gap (chromatid and chromosomal type), break (chromatid and chromosomal type), fragment and endomitosis (Plate,1). The aberrant cells showed more than one aberration were more frequently encountered in the bone marrow cells of rabbits exposed to AFB₁ comparing to other groups. The recorded numerical aberrations were in the form of peridiploidy which had higher frequency in G3 compared to other groups. AFB₁ is an indirect mutagen, its mutagenic activity produced by the metabolite aflatoxin 8, 9-epoxide which is formed from oxidation of AFB₁ by cytochrome P450 monooxidases. This metabolite binds to DNA and forms covalent adduct which disturbs DNA replication causing chromosomal aberrations. (Guengerich et al. 1994). Many authors recorded the mutagenic activity of AFB₁; Anwar et al. (1994) found a significant increase in chromosomal aberrations in rats' bone marrow cells treat-

ed with aflatoxins at a dose of 0.1ug AFB₁ /g b.wt. Also, Salassidis et al. (1991) observed a marked elevation in the structural chromosomal aberrations and sister chromatid exchange in rat and mouse hepatocyte. Supplementation of the probiotic *Pediococcus acidilactici* to the aflatoxicated diet inhibited the mutagenic effect of aflatoxin as it significantly decreased the frequency of total chromosomal aberrations (17.07 ± 1.53) compared to aflatoxicated group (33.34 ± 1.33). Our results are in accordance with Renner and Munzner (1991) who found that lactic acid bacteria has a strong anticlastogenic potential. It significantly reduces the chromosomal damage by about 80% in rodents exposed to the direct acting mutagen busulfan.

The mechanism of antimutagenicity of the probiotic might be due to the binding effect of lactic acid bacteria to several aflatoxins including AFB₁ (Gratz et al. 2006). The active antimutagenic substance is suspected to reside in the cell wall of the lactic acid bacteria. Therefore, it has been suggested that AFB₁ binds to bacterial cell wall or to the cell wall components rather than metabolic degradation (Peltonen et al. 2001). Bueno et al. (2007) mentioned that the binding of AFB₁ appears to occur on the bacterial surface of the lactic acid bacteria predominantly by hydrophobic interactions between the AFB₁ molecules and the carbohydrate and protein components of the bacterial cell wall resulting in the formation of a reversible complex between the toxin and

microorganism surface.

Results of erythrogram of different experimental groups are shown in table (2). The obtained data revealed non significant changes in hemoglobin concentration, hematocrit or erythrocytic count throughout the experimental period between the different groups. Similar results were obtained by Marine et al. (2002) who recorded non significant changes in total red blood cells in weanling piglets fed aflatoxicated diet. Also, Danicke et al. (2003) observed that ingestion of aflatoxin contaminated diet induced no effect on hemoglobin concentration in broilers. In contrast, Edrington et al (1997) reported an increase in hemoglobin concentration in broiler chicks fed diet containing aflatoxin (4mg /kg) diet.

Data of leukogram of different experimental groups are illustrated in table (3). Significant leukocytosis associated with neutrophilia and monocytosis was observed in G3 on the 2nd week post AFB₁ exposure in comparable to control group. These changes may be ascribed to the inflammatory response elicited by the aflatoxins (Kececi et al. 1998). These findings agree with Harvey et al. (1995) and Oguz et al. (2002). On the contrary, significant leukopenia associated with lymphopenia was observed in G3 on the 6th week of experiment. The observed lymphopenia may be resulted from the suppressive effects of aflatoxin on the immune response of rabbit. Our results are confirmed histopathologically as spleen and thymus

of aflatoxicated rabbit showed medullary lymphocytic depletion (Fig., 5&7). These results are in agreement with Raisuddin et al. (2006) who reported a marked decline in the total leukocytic count in AFB₁ treated weanling rat at a dose of 350 ug/kg b.wt. Moreover, Basmacioglu et al. (2005) recorded significant suppression in lymphocyte count in growing broiler chicken fed aflatoxicated diet (2 mg/kg diet) from 1 to 21 days of age.

Addition of the probiotic *Pediococcus acidilactici* to aflatoxin contaminated diet ameliorated the adverse effect of AFB₁ on the hematological parameters. Significant elevation in the total leukocytic and lymphocytic count was observed in (G4) on the 6th week of experiment in comparable to (G3). These changes may be referred to the immunomodulatory effect of the *Pediococcus acidilactici* (Leedle, 2000 and Silva, 2000). Similar results were obtained by Agawane and Lonkar (2004) who detected that the probiotic *Saccharomyces boulardii* was effective in reducing hematological alterations induced by ochratoxin in broilers.

Biochemical analysis of different experimental groups is shown in tables (4&5). The present data displayed a marked increase in the activities of serum ALT, AST and GGT of aflatoxicated rabbit (G3) from the 2nd week post aflatoxin exposure till the end of experiment in comparable to control group. The increment of these enzymes

reflects the hepatotoxic effect of the AFB₁ and damaged liver cells with subsequent release of enzymes into blood stream. Wogan, (1973) stated that AFB₁ is the most abundant and potent hepatotoxins. Moreover, the elevation of serum GGT activity suggested hepatic necrosis, thickness of bile duct and intrahepatic cholestasis (Kaneko, 1997). The obtained results are supported by the histopathological finding of the liver. Liver of aflatoxicated rabbit showed cholangitis and thickening in the wall of bile duct associated with leucocytic cells infiltration (Fig., 1). Our findings coincide with Harvey et al. (1991) in lambs, Mehta et al. (1993) in rats and Giroir et al. (1991) in broiler. Supplementation of aflatoxicated diet with the probiotic *Pediococcus acidilactici* (G4) considerably reduced the level of hepatic enzymes in comparable to (G3).

Significant increase of BUN was recorded in (G3) compared to control group. However serum creatinine level was non significantly changed in all experimental groups. The observed elevation of BUN without elevation of serum creatinine may be referred to the increase of protein catabolism as result of stress condition resulted from aflatoxin exposure. In addition this finding may denote mild nephrotoxic effect of AFB₁ as creatinine level may remain unchanged till one half to two thirds of the functioning units of both kidneys are destroyed (Allston, 1993). Such changes were relevant with the histopathological findings. The kidneys of aflatoxicated rabbits only

showed hypercellularity of the glomerular tufts while the kidneys of rabbits fed on aflatoxicated diet supplemented with *Pediococcus acidilactici* showed apparently normal renal tissues (Fig. 3&4). Our findings are parallel with Glahn et al. (1991) and Harvey et al. (1993). Values of BUN were significantly decreased in (G4) which is concurrently fed aflatoxicated diet supplemented with *Pediococcus acidilactici* compared to aflatoxicated group.

Concerning serum proteins profile, significant hypoproteinemia and hypoalbuminemia were recorded in the group of rabbits fed AFB₁ contaminated diet on the 4th and 6th week of experiment. These changes may be ascribed to the binding effect of AFB₁ to RNA and DNA of the cells resulting in disruption of transcription process and inhibition of protein synthesis (Yu, 1981). Moreover, it could be attributed to the damaging effect of AFB₁ on liver cells as confirmed by increased activities of ALT, AST and GGT and histopathological finding. Similar findings are obtained by Youssef et al. (2003) in rabbits, Kececi et al. (1998), Oguz et al. (2002) and Basmcioglu et al. (2005) in broilers. Hypoglobulinemia with decreased alpha, beta and gamma globulins were recorded in G3 on the 6th week of the experiment. Alpha and Beta globulins are mainly synthesized by the liver (Kaneko, 1997) and their decreased levels may be referred to the liver damage induced by AFB₁ (Tietz, 1996). The decreased level of the gamma globulin may be attributed to the

immunosuppressive effect of the AFB₁. This result is supported by lymphopenia in the peripheral blood and lymphocytic depletion in the spleen of aflatoxicated rabbits. Similar results were obtained by Osuna and Edds (1982) in pigs and Madheswaran et al. (2004) in chickens.

Improvement of serum proteins values and its fractions were noticed in (G4) fed aflatoxicated diet mixed with *Pediococcus acidilactici* which indicated increased protein synthesis.

From the measured biochemical parameters, it is clearly that *Pediococcus acidilactici* alleviated the harmful effects of AFB₁. It significantly reduced the elevated hepatic enzymes activities (ALT, AST and GGT) and BUN concentration. Moreover, it improved the values of serum proteins and its fractions. This effect may be referred to the blocking of AFB₁ intestinal absorption by *Pediococcus acidilactici* (El-Nezami et al. 2006 and Bueno et al. 2007).

Phagocytic percent and phagocytic index of neutrophils of rabbits received aflatoxin only revealed significant decrease compared to control group (Table, 6). These results agree with many investigations, Mohiuddin et al. (1986) recorded decreased phagocytic activity during aflatoxicosis in poultry. Ghosh et al. (1990) observed significant decrease in number of T-cells, albumin and globulins values in broiler under experimental aflatoxicosis. Moreover, Klebanoff and Clark (1978) mentioned that defects of phagocytosis often accompany cellular defects of chemotaxis,

impaired opsonization of particles by serum, deficiency of complement components and immunoglobulins. Sillvotti et al. (1997) and Mocchegiani et al. (1998) reported that aflatoxins-intoxicated pigs have reduced complement titers, decreased blastogenesis, decreased macrophage activation and depressed delayed hypersensitivity.

Rabbits received aflatoxin and probiotic exhibited significant increase in phagocytic and phagocytic index compared with rabbits received aflatoxin only while non significant difference were observed in these parameters when compared with control group. Addition of probiotic to this group ameliorates the adverse effect of aflatoxin which could be attributed to the reduction of dietary antigen load and enhancement of humoral and cellular immune response leading to protection. Fioramonti et al. (2003) summarized the main action of probiotics as a reinforcement of the intestinal mucosal barrier against deleterious agents and stimulate mucosal immunity. Similar results were obtained by Lee et al. (2007) who demonstrated that *pediococcus acidilactici*-based probiotic effectively enhances the resistance of birds and partially protects against negative growth effects with coccidiosis.

Finally we could conclude that in aflatoxin contaminated food, the use of the probiotic *pediococcus acidilactici* could be an alternative in field condition to reduce adverse effect and economic losses of aflatoxins.

Table (1): Effect of the probiotic *Pediococcus acidilactici* on the frequency of chromosomal aberrations of bone marrow cells of aflatoxicated rabbits.

Groups	M.NO	Structural aberrations									N.A.	TSA	TCA
		C.A.	Del.	Gap		Break		Frg.	Endom.	M.A.	Peridp.		
				Cr. gap	Crs. gap	Cr. B.	Crs. B.						
G.(1)	250	2.00 ±0.18 ^c	1.33 ±0.33 ^c	1.33 ±0.33 ^b	0.33 ± 0.03 ^b	0.00 ±0.00 ^c	0.00 ±0.00 ^b	0.67 ±0.08 ^c	0.33 ±0.06 ^b	0.33 ±0.08 ^b	2.33 ±0.33 ^b	6.00 ±0.40 ^c	8.33 ±0.45 ^c
G.(2)	250	3.00 ±0.58 ^{bc}	1.67 ±0.30 ^{bc}	1.00 ±0.01 ^b	0.00 ±0.00 ^b	0.00 ±0.00 ^c	0.00 ±0.00 ^b	0.33 ±0.03 ^c	0.33 ±0.13 ^b	0.67 0.08 ^b	3.33 ±0.30 ^{ab}	6.33 ±0.25 ^c	10.00 ±0.58 ^c
G.(3)	250	9.00 ±0.58 ^a	6.33 ±1.45 ^a	3.00 ±0.28 ^a	1.00 ±0.05 ^a	2.67 ±0.33 ^a	1.67 ±0.30 ^a	2.00 0.16 ^a	2.67 ±0.33 ^a	2.00 ±0.28 ^a	5.00 ±0.58 ^a	28.34 ±0.88 ^a	33.34 ±1.33 ^a
G.(4)	250	4.67 ±0.33 ^b	4.33 ±0.88 ^{ab}	2.00 ±0.05 ^{ab}	0.33 ±0.03 ^b	1.00 ±0.01 ^b	0.00 ±0.00 ^b	0.67 0.05 ^b	0.40 ±0.10 ^b	2.00 ±0.25 ^a	3.67 ±0.27 ^b	13.40 ±0.85 ^b	17.07 ±1.53 ^b

Values represent means ± SE

^{a-c}Values with different letters at the same column are significantly different at P<0.05

C.A., Centromeric Attenuation

Del., Deletion

Endom., Endomitosis

NA., Numerical Aberrations

Peridp., Peridiploidy

M.A., More than one aberration

TSA, Total structural aberrations

TCA, Total chromosomal aberrations

Table (2): Effect of the probiotic *Pediococcus acidilactici* on erythrogram of aflatoxicated rabbits

weeks	Parameters Groups	Hb (g/dl)	PCV (%)	RBCs ($\times 10^6/\mu\text{l}$)
2	G.(1)	13.13 \pm 0.93 ^a	31.33 \pm 2.03 ^a	5.25 \pm 0.46 ^a
	G.(2)	13.45 \pm 0.47 ^a	31.00 \pm 0.58 ^a	5.88 \pm 0.18 ^a
	G.(3)	16.87 \pm 1.12 ^a	31.90 \pm 1.16 ^a	5.83 \pm 0.19 ^a
	G.(4)	15.17 \pm 1.79 ^a	28.90 \pm 1.00 ^a	6.65 \pm 0.20 ^a
4	G.(1)	12.30 \pm 1.02 ^a	29.00 \pm 1.73 ^a	5.70 \pm 0.35 ^a
	G.(2)	14.17 \pm 0.47 ^a	30.67 \pm 0.88 ^a	5.37 \pm 0.33 ^a
	G.(3)	11.53 \pm 0.84 ^a	29.70 \pm 2.03 ^a	5.66 \pm 0.26 ^a
	G.(4)	11.43 \pm 1.15 ^a	29.37 \pm 1.77 ^a	5.70 \pm 0.37 ^a
6	G.(1)	13.43 \pm 0.75 ^a	31.33 \pm 2.03 ^a	5.50 \pm 0.12 ^a
	G.(2)	13.73 \pm 0.41 ^a	31.00 \pm 0.58 ^a	5.50 \pm 0.27 ^a
	G.(3)	12.23 \pm 0.37 ^a	31.00 \pm 1.16 ^a	5.33 \pm 0.18 ^a
	G.(4)	12.77 \pm 0.22 ^a	31.00 \pm 1.10 ^a	5.35 \pm 0.35 ^a

Values represent means \pm SE

^{a,b,c} Values with different letters at the same column are significant different at $p < 0.05$

Table (3): Effect of the probiotic *Pediococcus acidilactici* on leukogram of aflatoxicated rabbits

weeks	Parameters Groups	TLC ($\times 10^3/\mu\text{l}$)	Neutrophil ($\times 10^3/\mu\text{l}$)	Lymphocyte ($\times 10^3/\mu\text{l}$)	Esinophil ($\times 10^3/\mu\text{l}$)	Basophil ($\times 10^3/\mu\text{l}$)	Monocyte ($\times 10^3/\mu\text{l}$)
2	G.(1)	6.50 \pm 0.40 ^b	2.28 \pm 0.09 ^b	3.07 \pm 0.13 ^b	0.21 \pm 0.02 ^a	0.08 \pm 0.02 ^a	1.13 \pm 0.01 ^b
	G.(2)	8.13 \pm 0.15 ^{ab}	2.28 \pm 0.12	4.44 \pm 0.09 ^a	0.25 \pm 0.04 ^a	0.08 \pm 0.04 ^a	1.12 \pm 0.04 ^{ab}
	G.(3)	9.23 \pm 0.51 ^a	4.60 \pm 0.41 ^a	3.87 \pm 0.10 ^b	0.25 \pm 0.02 ^a	0.10 \pm 0.04 ^a	0.77 \pm 0.07 ^a
	G.(4)	7.58 \pm 0.95 ^{ab}	2.64 \pm 0.26 ^b	4.53 \pm 0.33 ^{ab}	0.14 \pm 0.04 ^a	0.08 \pm 0.01 ^a	1.11 \pm 0.01 ^a
4	G.(1)	9.65 \pm 1.28 ^a	3.30 \pm 0.40 ^a	3.11 \pm 0.52 ^a	0.42 \pm 0.09 ^a	0.25 \pm 0.03 ^a	0.83 \pm 0.08 ^a
	G.(2)	9.93 \pm 0.64 ^a	3.33 \pm 0.20 ^a	3.11 \pm 0.50 ^a	0.21 \pm 0.02 ^a	0.10 \pm 0.02 ^a	0.91 \pm 0.04 ^a
	G.(3)	8.63 \pm 0.26 ^a	3.06 \pm 0.51 ^a	4.65 \pm 0.26 ^a	0.34 \pm 0.02 ^a	0.10 \pm 0.03 ^a	0.84 \pm 0.05 ^a
	G.(4)	9.22 \pm 0.84 ^a	3.40 \pm 0.27 ^a	3.01 \pm 0.35 ^a	0.36 \pm 0.01 ^a	0.08 \pm 0.02 ^a	0.81 \pm 0.01 ^a
6	G.(1)	9.07 \pm 0.81 ^a	3.40 \pm 0.31 ^a	3.09 \pm 0.24 ^a	0.21 \pm 0.05 ^a	0.10 \pm 0.05 ^a	0.84 \pm 0.04 ^a
	G.(2)	8.88 \pm 0.51 ^a	3.78 \pm 0.30 ^a	4.50 \pm 0.33 ^a	0.20 \pm 0.02 ^a	0.20 \pm 0.01 ^a	0.78 \pm 0.02 ^a
	G.(3)	6.05 \pm 0.87 ^b	2.79 \pm 0.2 ^a	2.26 \pm 0.22 ^c	0.33 \pm 0.05 ^a	0.27 \pm 0.08 ^a	1.31 \pm 0.02 ^a
	G.(4)	8.60 \pm 0.25 ^a	3.91 \pm 0.26	3.75 \pm 0.32 ^b	0.26 \pm 0.06 ^a	0.28 \pm 0.02 ^a	0.86 \pm 0.11 ^a

Values represent means \pm SE

^{a,b,c} Values with different letters at the same column are significant different at $p < 0.05$

Table (4): Effect of the probiotic *Pediococcus acidilactici* on some serum biochemical parameters of aflatoxicated rabbits

weeks	Parameters	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
	Groups					
2	G.(1)	25.67 ± 2.41 ^c	57.33 ± 1.45 ^{bc}	6.83 ± 0.20 ^b	19.00 ± 2.08 ^c	0.95 ± 0.02 ^a
	G.(2)	27.00 ± 1.00 ^c	52.83 ± 1.48 ^c	6.67 ± 0.42 ^b	19.67 ± 0.88 ^c	0.93 ± 0.04 ^a
	G.(3)	39.67 ± 1.20 ^a	70.03 ± 1.29 ^a	12.83 ± 1.63 ^a	33.33 ± 1.86 ^a	0.87 ± 0.04 ^a
	G.(4)	32.67 ± 1.77 ^b	61.75 ± 2.04 ^b	10.30 ± 1.39 ^{ab}	24.20 ± 2.08 ^b	0.88 ± 0.04 ^a
4	G.(1)	29.33 ± 1.77 ^c	55.53 ± 0.74 ^{bc}	8.40 ± 0.59 ^c	18.00 ± 1.16 ^c	0.92 ± 0.02 ^a
	G.(2)	28.00 ± 2.31 ^c	52.00 ± 1.16 ^c	7.80 ± 0.57 ^c	20.00 ± 1.16 ^c	0.91 ± 0.02 ^a
	G.(3)	59.67 ± 2.61 ^a	75.37 ± 1.71 ^a	25.33 ± 1.45 ^a	35.00 ± 1.53 ^a	0.87 ± 0.03 ^a
	G.(4)	44.00 ± 3.22 ^b	57.73 ± 1.46 ^b	13.67 ± 0.88 ^b	27.67 ± 1.45 ^b	0.86 ± 0.06 ^a
6	G.(1)	31.00 ± 2.31 ^c	53.67 ± 2.49 ^{bc}	7.93 ± 0.18 ^c	19.67 ± 0.33 ^c	0.89 ± 0.04 ^a
	G.(2)	30.00 ± 2.52 ^c	51.00 ± 2.08 ^c	8.40 ± 0.49 ^c	19.57 ± 1.20 ^c	0.93 ± 0.01 ^a
	G.(3)	57.33 ± 2.34 ^a	80.00 ± 1.45 ^a	30.33 ± 1.45 ^a	32.00 ± 1.16 ^a	0.86 ± 0.03 ^a
	G.(4)	47.33 ± 1.77 ^b	60.47 ± 4.25 ^b	18.00 ± 1.16 ^b	24.67 ± 1.77 ^b	0.87 ± 0.08 ^a

Values represent means ± SE

^{a-c} Values with different letters at the same column are significantly different at $p < 0.05$

Table (5): Effect of the probiotic *Pediococcus acidilactici* on proteins profile and its fractions of aflatoxicated rabbits

weeks	Parameters	T.proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	α .Globulins (g/dl)	β .Globulins (g/dl)	δ .Globulins (g/dl)	A/G Ratio
	Groups							
2	G.(1)	7.02 ± 0.14 ^a	3.57 ± 0.23 ^a	3.45 ± 0.46 ^a	1.00 ± 0.04 ^a	1.00 ± 0.06 ^a	1.45 ± 0.13 ^a	1.03 ± 0.10 ^a
	G.(2)	7.13 ± 0.09 ^a	3.27 ± 0.12 ^a	3.83 ± 0.23 ^a	1.13 ± 0.07 ^a	1.10 ± 0.17 ^a	1.60 ± 0.19 ^a	0.85 ± 0.06 ^a
	G.(3)	6.58 ± 0.21 ^a	3.04 ± 0.08 ^a	3.54 ± 0.16 ^a	1.03 ± 0.04 ^a	1.11 ± 0.03 ^a	1.40 ± 0.12 ^a	0.86 ± 0.06 ^a
	G.(4)	6.90 ± 0.12 ^a	3.17 ± 0.17 ^a	3.73 ± 0.15 ^a	1.07 ± 0.02 ^a	1.12 ± 0.14 ^a	1.54 ± 0.08 ^a	0.85 ± 0.10 ^a
4	G.(1)	7.48 ± 0.09 ^a	3.72 ± 0.12 ^a	3.76 ± 0.22 ^a	1.18 ± 0.12 ^{ab}	1.08 ± 0.09 ^a	1.50 ± 0.08 ^a	0.98 ± 0.13 ^a
	G.(2)	7.58 ± 0.11 ^a	3.50 ± 0.17 ^{ab}	4.08 ± 0.28 ^a	1.20 ± 0.14 ^a	1.18 ± 0.01 ^a	1.62 ± 0.21 ^a	0.85 ± 0.05 ^b
	G.(3)	6.47 ± 0.12 ^b	3.07 ± 0.24 ^b	3.40 ± 0.41 ^a	0.96 ± 0.04 ^b	1.04 ± 0.04 ^a	1.40 ± 0.14 ^a	0.90 ± 0.08 ^{ab}
	G.(4)	6.63 ± 0.19 ^b	3.13 ± 0.11 ^b	3.50 ± 0.20 ^a	1.10 ± 0.06 ^b	1.00 ± 0.11 ^a	1.45 ± 0.04 ^a	0.89 ± 0.06 ^{ab}
6	G.(1)	7.83 ± 0.13 ^a	3.82 ± 0.17 ^{ab}	4.01 ± 0.11 ^a	1.22 ± 0.06 ^{ab}	1.19 ± 0.03 ^a	1.60 ± 0.12 ^a	0.95 ± 0.08 ^a
	G.(2)	8.36 ± 0.07 ^a	4.00 ± 0.09 ^a	4.36 ± 0.08 ^a	1.30 ± 0.07 ^a	1.21 ± 0.05 ^a	1.85 ± 0.19 ^a	0.92 ± 1.11 ^a
	G.(3)	5.49 ± 0.15 ^c	2.81 ± 0.26 ^c	2.68 ± 0.24 ^c	0.60 ± 0.03 ^c	0.98 ± 0.11 ^b	1.10 ± 0.23 ^b	1.04 ± 1.10 ^a
	G.(4)	6.84 ± 0.25 ^b	3.20 ± 0.17 ^b	3.63 ± 0.25 ^b	1.01 ± 0.10 ^{bc}	1.19 ± 0.03 ^{ab}	1.44 ± 0.07 ^b	0.88 ± 0.10 ^a

Values represent means ± SE

^{a-c} Values with different letters at the same column are significantly different at $p < 0.05$

Table (6): Effect of the probiotic *Pediococcus acidilactici* on Phagocytic percentage and index of aflatoxicated rabbit neutrophils

Parameters Groups	Phagocytic %	Phagocytic index
G.(1)	57.5 ± 1.90 ^b	1.22 ± 0.02 ^b
G.(2)	66.0 ± 1.90 ^a	1.36 ± 0.03 ^a
G.(3)	44.0 ± 1.70 ^c	1.05 ± 0.02 ^c
G.(4)	53.0 ± 2.50 ^b	1.18 ± 0.05 ^b

Values represent means ± SE

^{a-c} Values with different letters at the same column are significantly different at $p < 0.05$

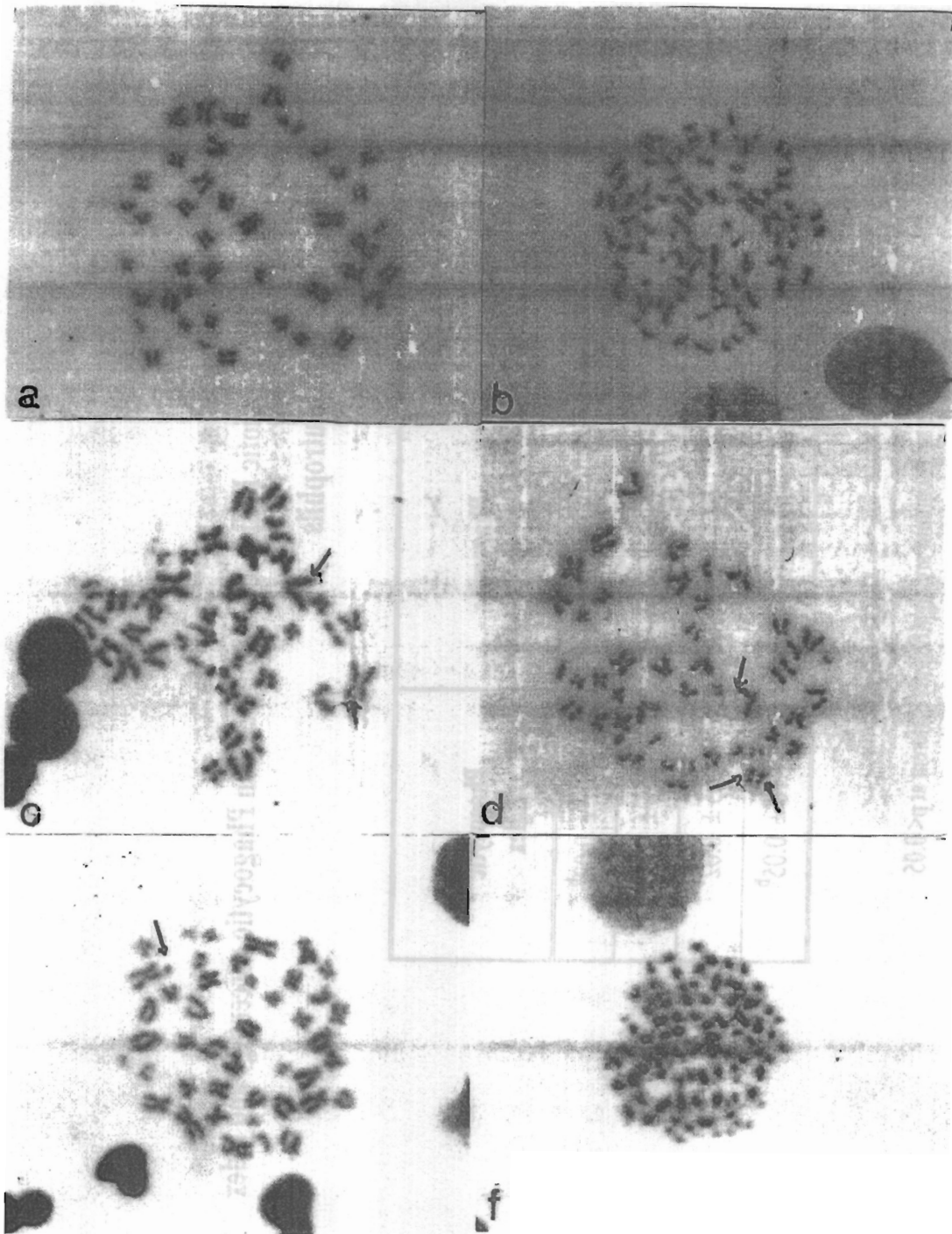


Plate (1): Different types of chromosomal aberrations in bone marrow cells of aflatoxicated rabbits
 a) Normal metaphase (b) Centromeric attenuation (c) Deletion and Chromatid gap (d) Chromosomal break and Deletion (e) Fragment (f) Endomitosis

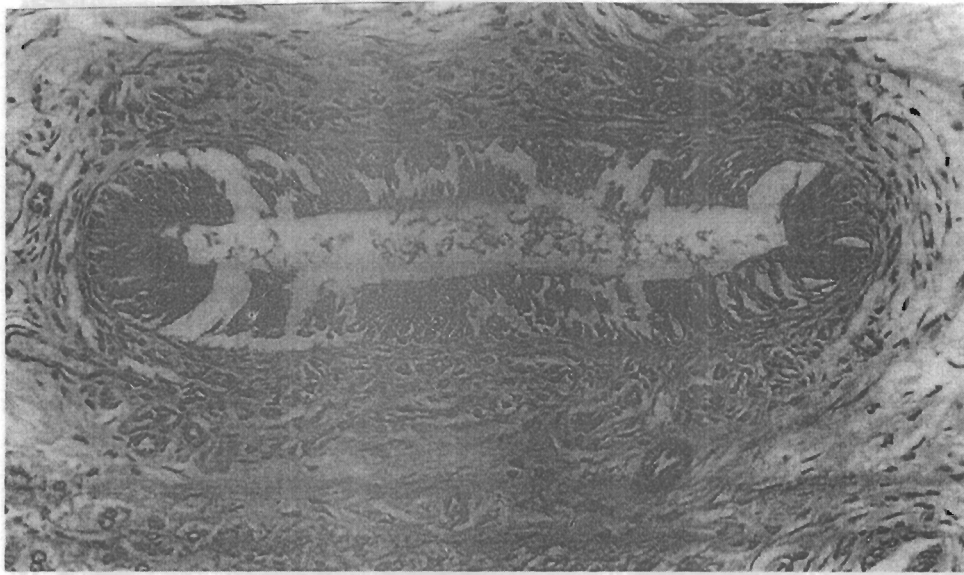


Fig. (1): Liver of aflatoxicated rabbit showing cholangitis and thickening in the wall of bile duct associated with leukocytic cells infiltration (H and E x 200).

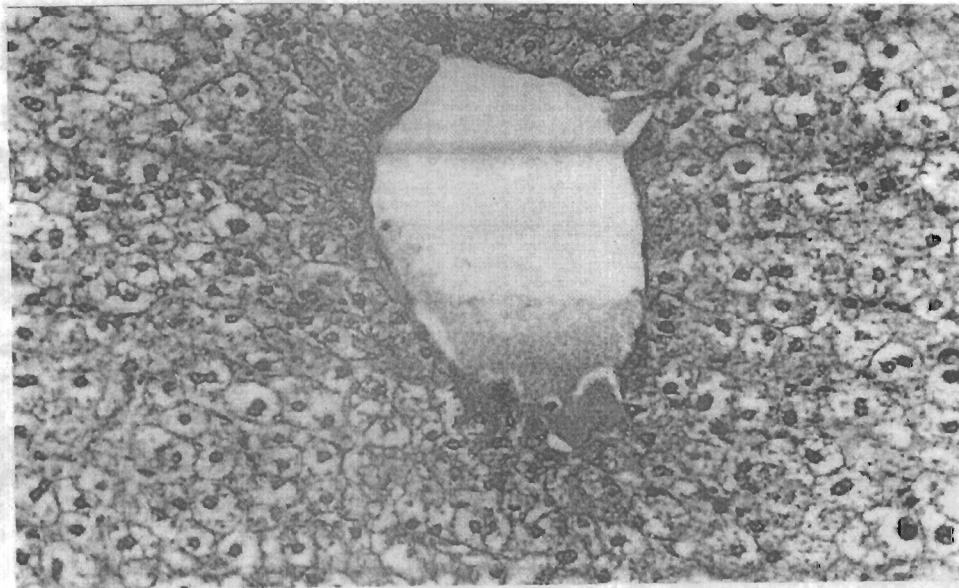


Fig. (2): Liver of rabbit fed on aflatoxicated diet supplemented with the probiotic *Pediococcus acidilactici* showing hydrobic degeneration of hepatocytes (H and E x 200).

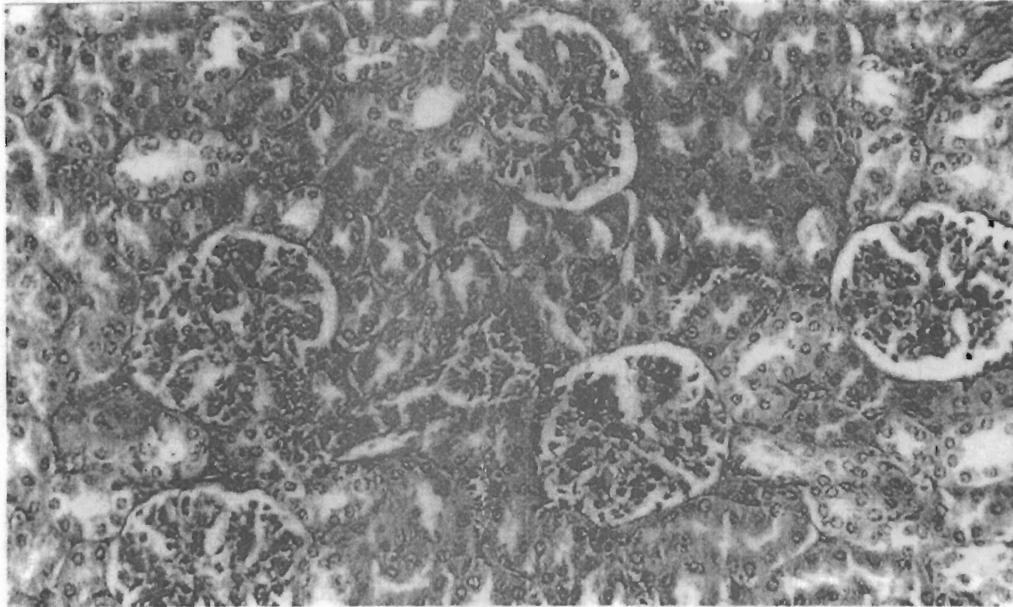


Fig. (3): Kidney of aflatoxicated rabbit showing hypercellularity of the glomerular tufts (H and E x 200).

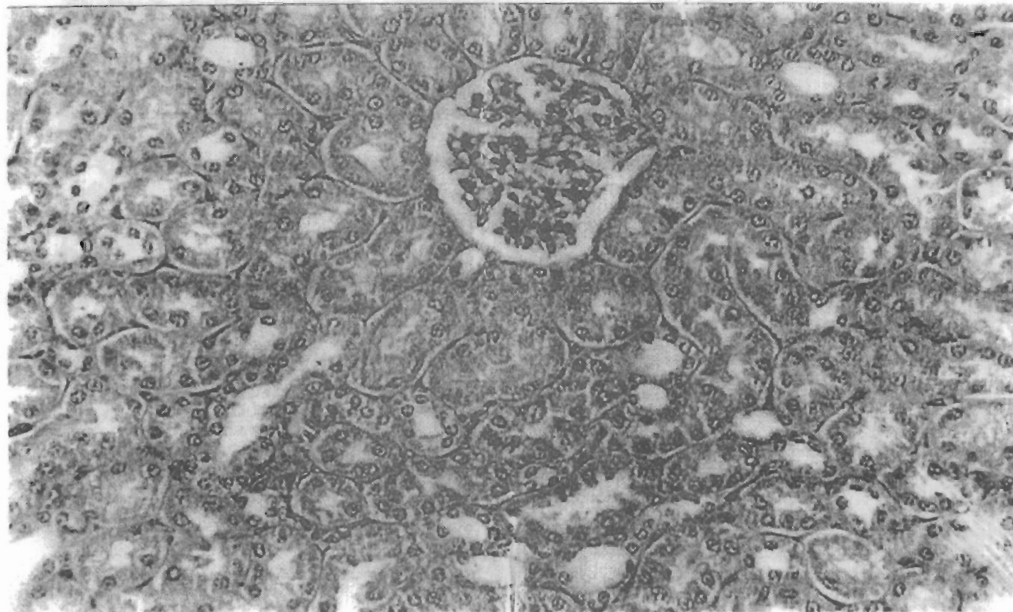


Fig. (4): Kidney of rabbit fed on aflatoxicated diet supplemented with the probiotic *Pediococcus acidilactici* showing apparent normal renal parenchyma (H and E x 200).

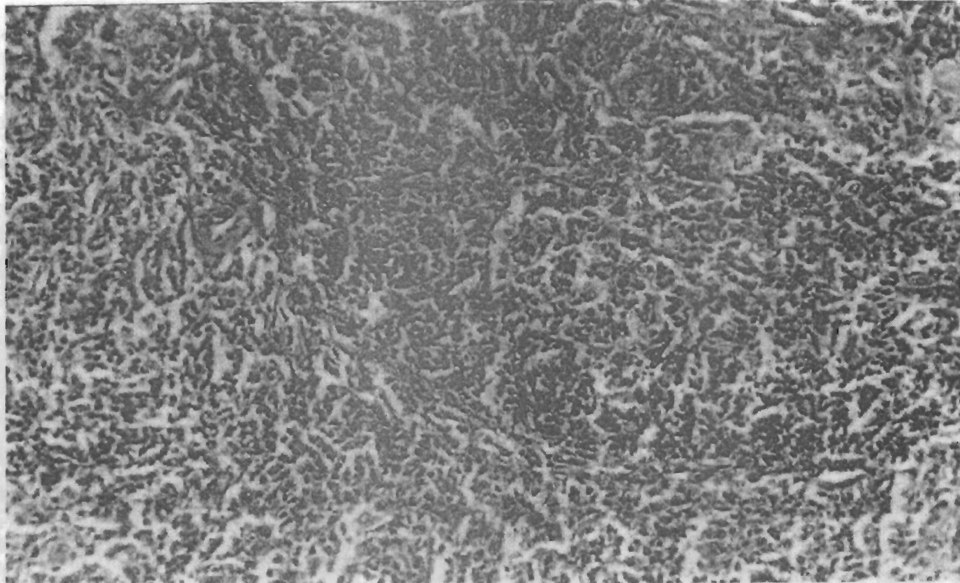


Fig. (5): Spleen of aflatoxicated rabbit showing slight lymphocytic depletion (H and E x 200).

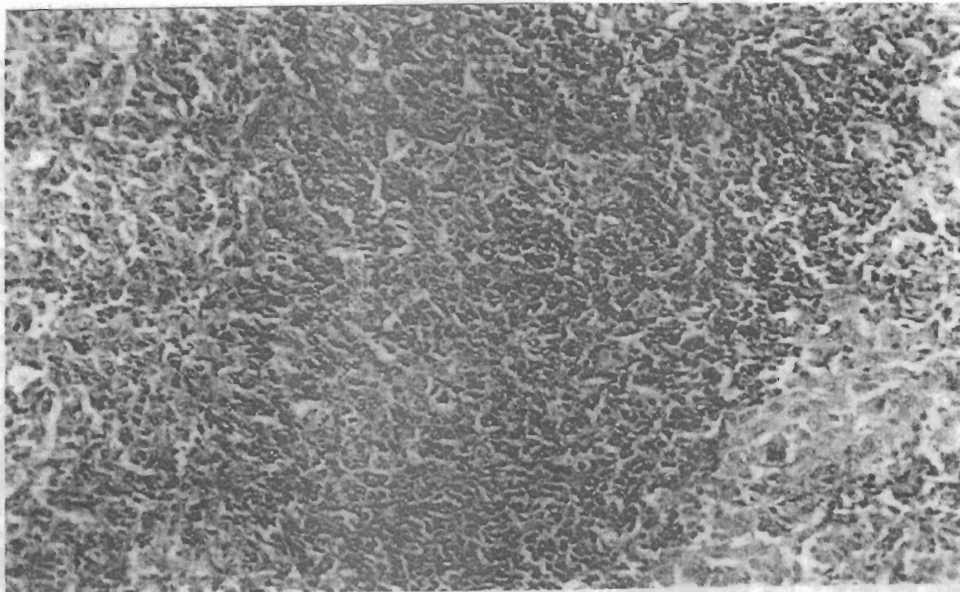


Fig. (6): Spleen of rabbit fed on aflatoxicated diet supplemented with the probiotic *Pediococcus acidilactici* showing no histopathological changes (H and E x 200).

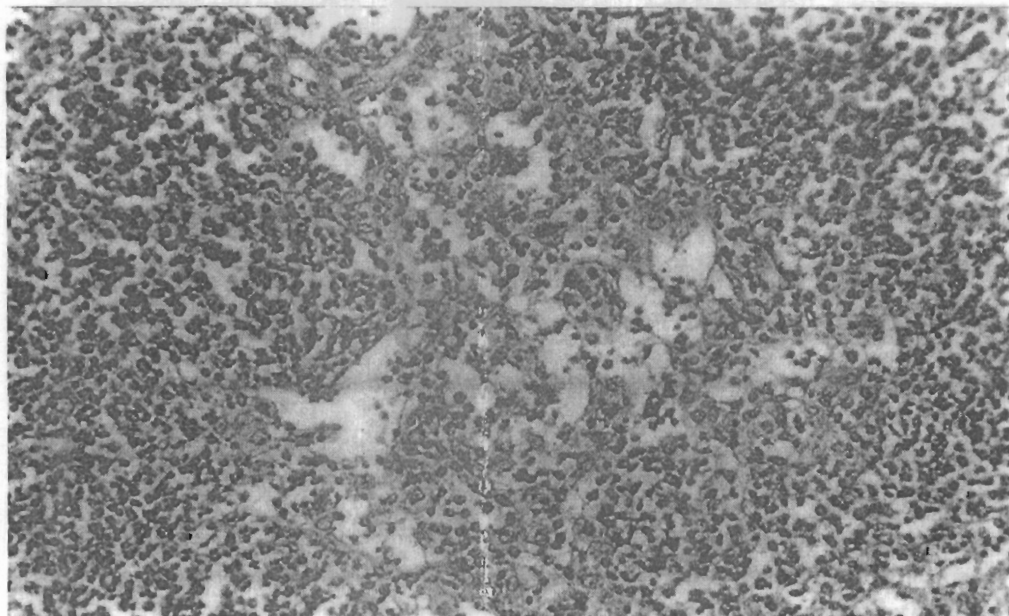


Fig. (7): Thymus of aflatoxicated rabbit showing medullary lymphocytic depletion (H and E x 200).

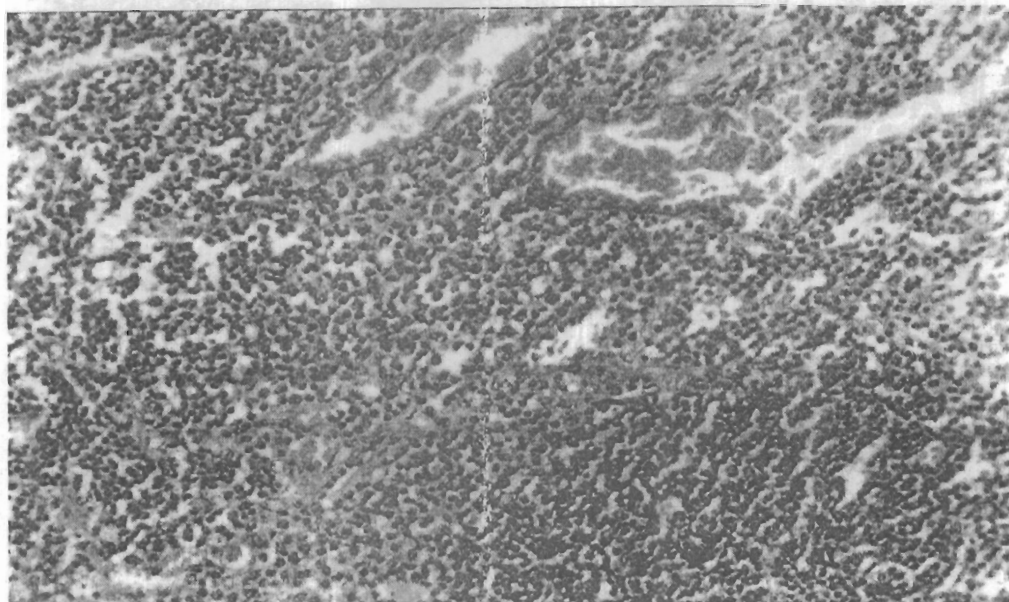


Fig. (8): Thymus of rabbit fed on aflatoxicated diet supplemented with the probiotic *Pediococcus acidilactici* showing congestion of blood vessels (H and E x 200).

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تأثير المحفز الحيوي بديوكوكاس اسيدوفلاس على الشذوذات الكروموسومية ،
التغيرات الباثولوجية الأكلينيكية و المناعية في ذكور الارانب المعرضة للافلا توكسين

ب،

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٤.٢ - قسم الكيمياء الحيوية وقسم المناعة - معهد بحوث صحة الحيوان - الدقى

اجريت هذه الدراسة على عدد اربعة وعشرون من الارانب النيوزيلاندى البيضاء بهدف دراسة تأثير المحفز الحيوي بديوكوكاس اسيدوفلاس على الشذوذات الكروموسومية لخلايا النخاع و صورة الدم وبعض مكونات السيرم و التغيرات الهستوباثولوجية لبعض الأنسجة وكذلك تاثيره على كفاءة خلايا النتروفيل و معدل الالتهام في الارانب المعرضة للافلا توكسين ب١.

تم تقسيم الارانب الي اربعة مجموعات، المجموعة الاولى استخدمت كمجموعة ضابطة، المجموعة الثانية تم اعطائها المحفز الحيوي بديوكوكاس اسيدوفلاس (100g/ton diet) لمدة 6 اسابيع، المجموعة الثالثة تم تعرضها للافلا توكسين ب١ عن طريق خلطها بالعلف - (60ug/kg diet) لمدة 6 اسابيع. اما المجموعة الرابعة فقد تم تعرضها للافلا توكسين ب١ عن طريق خلطه بالعلف (60ug/kg diet) بالاضافة الى المحفز الحيوي بديوكوكاس اسيدوفلاس (100g/ton diet) لمدة 6 اسابيع متتالية.

و بإجراء الفحص الوراثى الخلوى أظهرت النتائج ان المحفز الحيوي بديوكوكاس اسيدوفلاس قد

احدث قلة معنوية في عدد الخلايا التي تحتوي شذوذات كروموسومية.

و بفحص خلايا الدم لوحظ تحسن فى عدد كرات الدم البيضاء وخاصة خلايا الليمفوسيت و لقد أظهرت التغيرات البيوكيميائية للدم تحسن ملحوظ حيث انخفض نشاط الألائين امينو ترانسفيريز و الأسيرتات امينو ترانسفيريز و الجاما جلوتاميل ترانسفيراز بالإضافة إلى قلة معنوية في معدلات البولينا في الدم وكذلك تحسن معنوى فى تركيز البروتين الكلى و الألبومين و الجلوبيولين.

كما احدث المحفز الحيوي بديوكوكاس اسيدوفلاس زيادة معنوية فى كفاءة خلايا النتروفيل و معدل الالتهام في الارانب المعرضة للافلا توكسين ب١.