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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 B_1 (AFB_1) 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 AFB_1/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 contaminated with 60 ug of AFB_1/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 AFB_1 possesses a mutagenic effect. It significantly increases the frequency of chromosomal aberrations. AFB_1 induced significant decrease in the total leukocytic count associated with lympopenia. 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 AFB_1 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 B_1, chromosomal aberrations, hematobiochemical alterations.

INTRODUCTION

Mycotoxins are secondary metabolites of toxigenic moulds that represent a great risk for human and animals. Aflatoxin B_1 (AFB_1) 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 AFB_1 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 AFB_1 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 AFB_1 in male New-zealand white rabbits. We investigate the effect of Pediococcus acidilactici on chromosomal aberrations, clincopathological 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 B_1:

Aflatoxin B_1 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 adlibitum. 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 Pediococcus 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 ug of AFB_1/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 Pediococcus acidilactici (the same dose mentioned in G2) and artificially contaminated with 60 ug of AFB_1/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 Weicheselbaun (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 W (1972).

4- Phagocytic activity:

Phagocytic activity of neutrophils for cell me ed immune-response assessment using De 500.000 M.W. from Sigma according to the m od described by Wilkinson (1981).

5- Histopathological studies:

Specimens from liver, kidneys, spleen and t mus were taken directly after sacrification of t examined rabbits and fixed immediately in 10 neutral buffered formalin solution. Paraffin se tions of 4-6 µ thick were prepared and staine with hematoxylin and eosin (Bancroft et a 1996).

6- Statistical analysis:

Data were compared across groups using analysi of variance (ANOVA). Data were expressed a mean ± S.E. Level of significance of $P < 0.05$ wa chosen to identify the significant differences (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Aflatoxin B_1 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 AFB_1 in rabbits. Results of chromosomal aberrations of different experimental groups are shown in table (1). Cytogenetic analysis revealed that AFB_1 possesses a mutagenic effect as it significantly increases the frequency of both individual and total chromosomal aberrations of rabbits bone mallow 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 AFB_1 comparing to other groups. The recorded numerical aberrations were in the form of peridiploidy which had higher frequency in G3 compared to other groups. AFB_1 is an indirect mutagen, its mutagenic activity produced by the metabolite aflatoxin 8, 9-epoxide which is formed from oxidation of AFB_1 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 AFB_1; Anwar et al. (1994) found a significant increase in chromosomal aberrations in rats' bone marrow cells treated with aflatoxins at a dose of 0.1ug AFB_1 /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 AFB_1 (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 AFB_1 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 AFB_1 appears to occur on the bacterial surface of the lactic acid bacteria predominantly by hydrophobic interactions between the AFB_1 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 AFB_1 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 AFB_1 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 AFB_1 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 AFB_1 and damaged liver cells with subsequent release of enzymes into blood stream. Wogan, (1973) stated that AFB_1 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 AFB_1 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 AFB_1 contaminated diet on the 4^{th} and 6^{th} week of experiment. These changes may be ascribed to the binding effect of AFB_1 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 AFB_1 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 AFB_1 (Tietz, 1996). The decreased level of the gamma globulin may be attributed to the

immunosuppressive effect of the AFB_1. 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 AFB_1. 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 AFB_1 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, de-
ciency of complement components and immu-
globulins. Sillvotti et al. (1997) and Mocchegi-
et al. (1998) reported that aflatoxins-intoxicat-
pigs have reduced complement titers, decrease
blastogenesis, decreased macrophage activati
and depressed delayed hypersensitivity.

Rabbits received aflatoxin and probiotic exhibi-
ed significant increase in phagocytic and phago-
cytic index compared with rabbits received afl-
toxin only while non significant difference we-
observed in these parameters when compar-
with control group. Addition of probiotic to th-
group ameliorates the adverse effect of aflatoxi-
which could be attributed to the reduction of di-
tary antigen load and enhancement of humor-
and cellular immune response leading to protec-
tion. Fioramonti et al. (2003) summarized th-
main action of probiotics as a reinforcement o-
the intestinal mucosal barrier against deleterio-
agents and stimulate mucosal immunity. Simil-
results were obtained by Lee et al. (2007) wh-
demonstrated that pedicoccus acidilactici-base-
probiotic effectively enhances the resistance o-
birds and partially protects against negativ-
growth effects with coccidiosis.

Finally we could conclude that in afiatoxin con-
taminated food, the use of the probiotic pedicoc-
cus 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-cValues 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	Groups \ parameters	Hb (g/dl)	PCV (%)	RBCs (x10⁶/ul)
—	G.(1)	13.13 ± 0.93 [a]	[illegible]	[illegible]
2	G.(2)	13.45 ± 0.47 [a]	[illegible]	5.88 ± 0.18 [a]
	G.(3)	16.87 ± 1.12 [a]	31.00 ± 1.16 [a]	5.83 ± 0.10 [a]
	G.(4)	15.17 ± 1.79 [a]	28.00 ± 1.00 [a]	6.5 ± 0.20 [a]
4	G.(1)	12.30 ± 1.02 [a]	29.00 ± [illegible] [a]	[illegible]
	G.(2)	14.17 ± 0.47 [a]	30.67 ± 0.88 [a]	6.37 ± 0.33 [a]
	G.(3)	11.53 ± 0.84 [a]	29.[illegible] ± 2.03 [a]	5.66 ± 0.26 [a]
	G.(4)	[illegible]	[illegible]	[illegible]
6	G.(1)	13.43 ± 0.75 [a]	31.33 ± 2.03 [a]	[illegible]
	G.(2)	13.73 ± 0.41 [a]	32.00 ± 0.58 [a]	[illegible]
	G.(3)	12.33 ± 0.37 [a]	[illegible]	[illegible]
	G.(4)	[illegible]	[illegible]	6.35 [a]

Values represent means ± SE
[a] Values with different letters [illegible] the same column are [illegible] different at p< 0.05

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

weeks	Parameters \ Groups	TLC (x10³/µl)	Neutrophil (x10³/µl)	Lymphocyte (x10³/µl)	Esinophil (x10³/µl)	Basophil (x10³/µl)	Monocyte (x10³/µl)
2	G.(1)	6.50 ± 0.40 [b]	2.28 ± 0.09 [b]	[illegible] ± 0.18 [b]	[illegible] ± 0.02 [a]	0.08 ± 0.02 [a]	[illegible] ± 0.01 [b]
	G.(2)	8.13 ± 0.15 [ab]	[illegible]	[illegible] ± 0.09 [a]	[illegible] ± 0.04 [a]	[illegible]	[illegible] ± 0.04 [ab]
	G.(3)	9.23 ± 0.51 [a]	4.69 ± 0.41 [a]	[illegible] [b]	0.25 ± 0.02 [a]	0.10 ± [illegible] [a]	[illegible] ± 0.07 [a]
	G.(4)	7.58 ± 0.95 [ab]	2.64 ± 0.26 [b]	[illegible] ± 0.33 [ab]	0.14 ± 0.04 [a]	0.08 ± 0.01 [a]	[illegible] [a]
4	G.(1)	9.65 ± 1.28 [a]	3.30 ± 0.40 [a]	[illegible] ± 0.52 [a]	0.42 ± 0.09 [a]	0.25 ± 0.05 [a]	[illegible] 0.08 [a]
	G.(2)	9.93 ± 0.64 [a]	5.33 ± 0.20 [a]	[illegible] ± 0.50 [a]	0.21 ± 0.02 [a]	0.[illegible] ± 0.02 [a]	[illegible] ± 0.04 [a]
	G.(3)	8.63 ± 0.26 [a]	3.06 ± 0.51 [a]	4.65 ± 0.26 [a]	0.34 ± 0.02 [a]	0 ± 0.03 [a]	[illegible] ± 0.05 [a]
	G.(4)	9.22 ± 0.84 [a]	[illegible] ± 0.27 [a]	[illegible] ± 0.33 [a]	0.36 ± 0.01 [a]	[illegible] ± 0.02 [a]	[illegible] ± 0.01 [a]
6	G.(1)	[illegible] ± 0.81 [a]	[illegible] ± 0.31 [a]	[illegible] ± 0.24 [a]	[illegible] ± 0.05 [a]	[illegible] ± 0.05 [a]	[illegible] ± 0.04 [a]
	G.(2)	8.88 ± [illegible] [a]	[illegible] ± 0.30 [a]	4.50 ± 0.33 [a]	0.20 ± 0.02 [a]	0.[illegible] ± [illegible] [a]	[illegible] ± 0.02 [a]
	G.(3)	6.05 ± 0.87 [b]	2.79 ± 0.2[illegible] [a]	2.26 ± 0.22 [c]	[illegible] ± 0.05 [a]	[illegible] ± [illegible] [a]	[illegible] ± 0.02 [a]
	G.(4)	8.60 ± 0.25 [a]	3.91 ± 0.26	[illegible] ± 0.32 [b]	[illegible] ± 0.06 [a]	[illegible] ± 0.02 [a]	[illegible] [a]

Values represent means ± SE
[a] Values with different letters [illegible] the same column are significantly different [illegible]

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

weeks	Parameters / Groups	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
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 / Groups	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
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-cValues 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

Groups / Parameters	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 $p < 0.05$

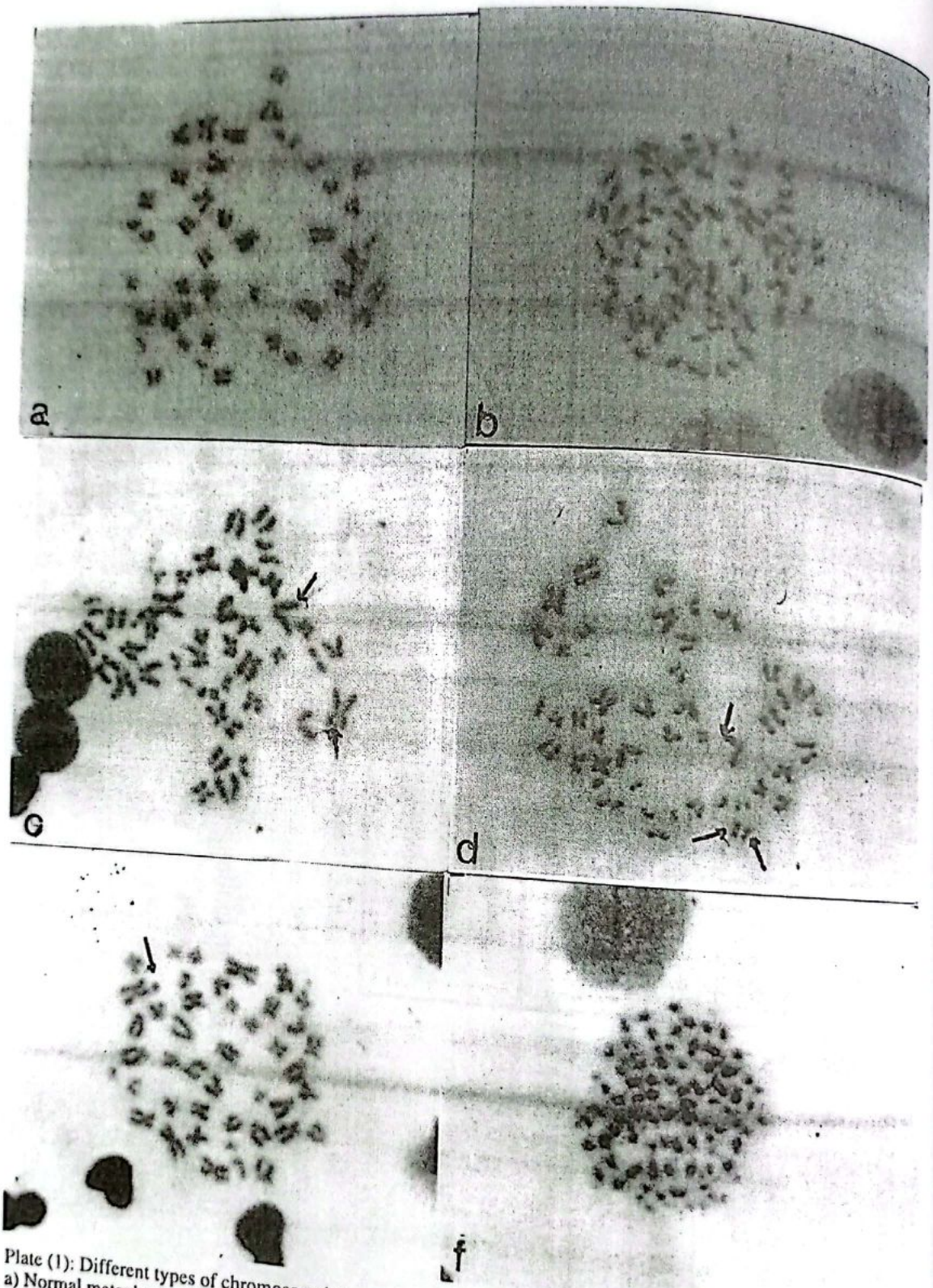

Plate (1): Different types of chromosomal aberrations in bone marrow cells of aflatoxicated rabbits
a) Normal metaphase (b) Centromeric attenuation (c) Deletion and Chromatid gap (d) Chromosomal break and Deletion (e) Fragment (f) Endomitosis.

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).

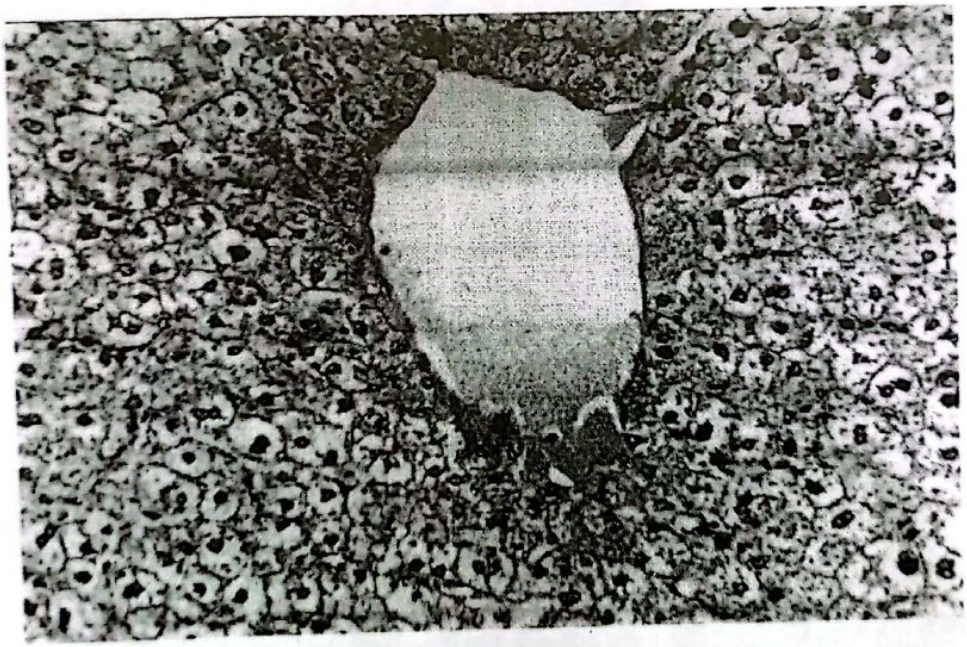

Fig. (2): Liver of rabbit fed on aflatoxicated diet supplemented with the probiotic Pediococcus acidilactici showing hydrobic degeneration of hepatocytes (H and E x 200).

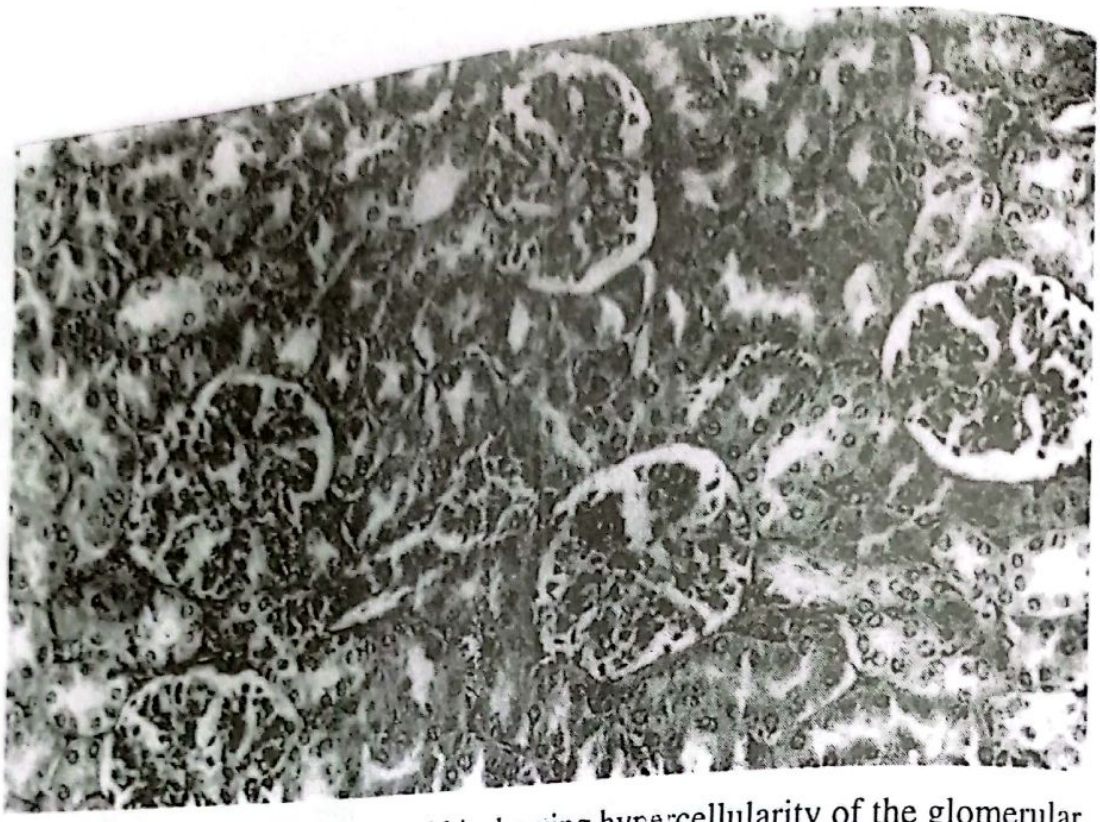

Fig. (3): Kidney of aflatoxicated rabbit showing hypercellularity of the glomerular tufts (H and E x 200).

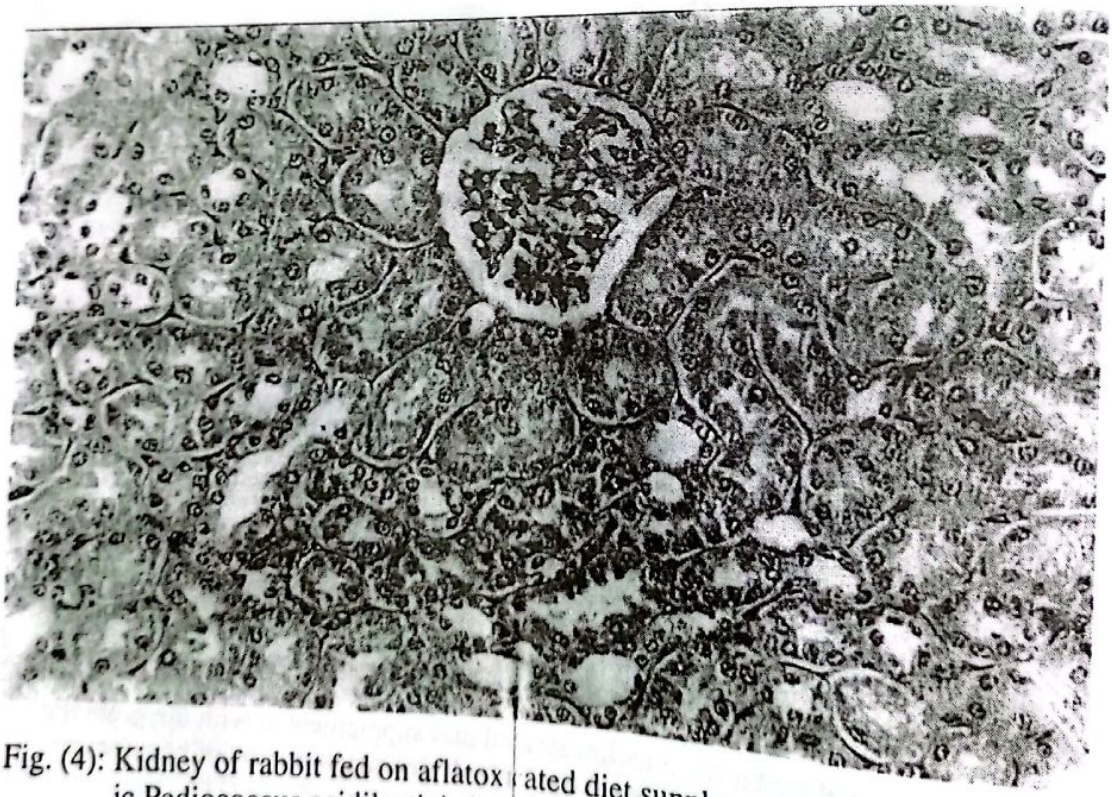

Fig. (4): Kidney of rabbit fed on aflatoxicated diet supplemented with the probiotic Pediococcus acidilactici showing apparent nromal renal parenchyma (H and E x 200).

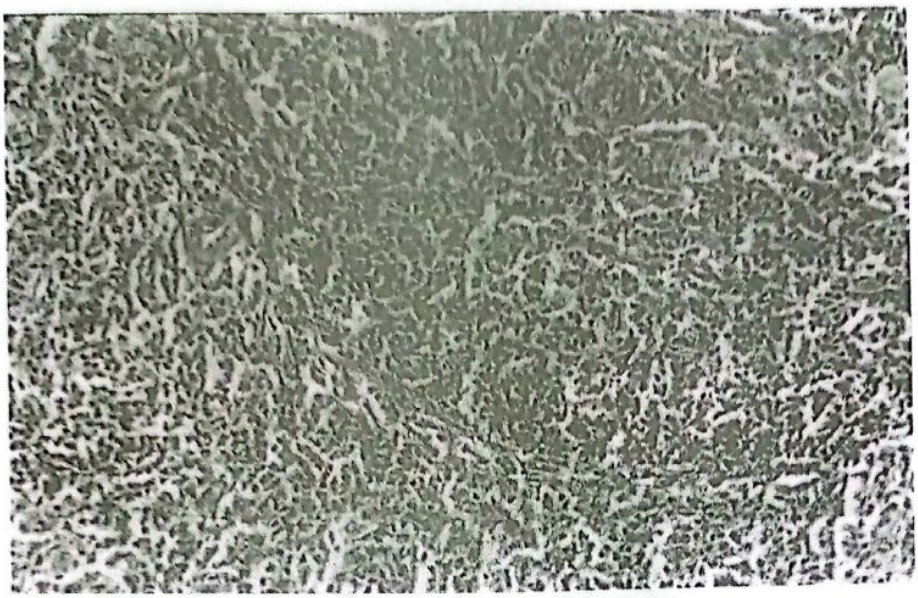

Fig. (5): Spleen of aflatoxicated rabbit showing slight lymphocytic depletion (H and E x 200).

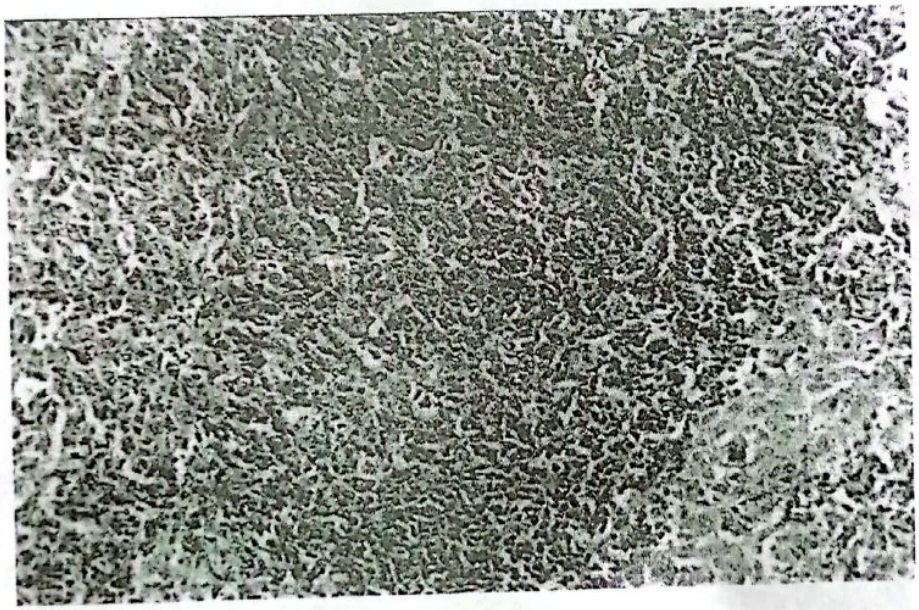

Fig. (6) :Spleen of rabbit fed on aflatoxicated diet supplemented with the probiotic Pediocooccus acidilactici showing no histopathological changes (H and E x 200).

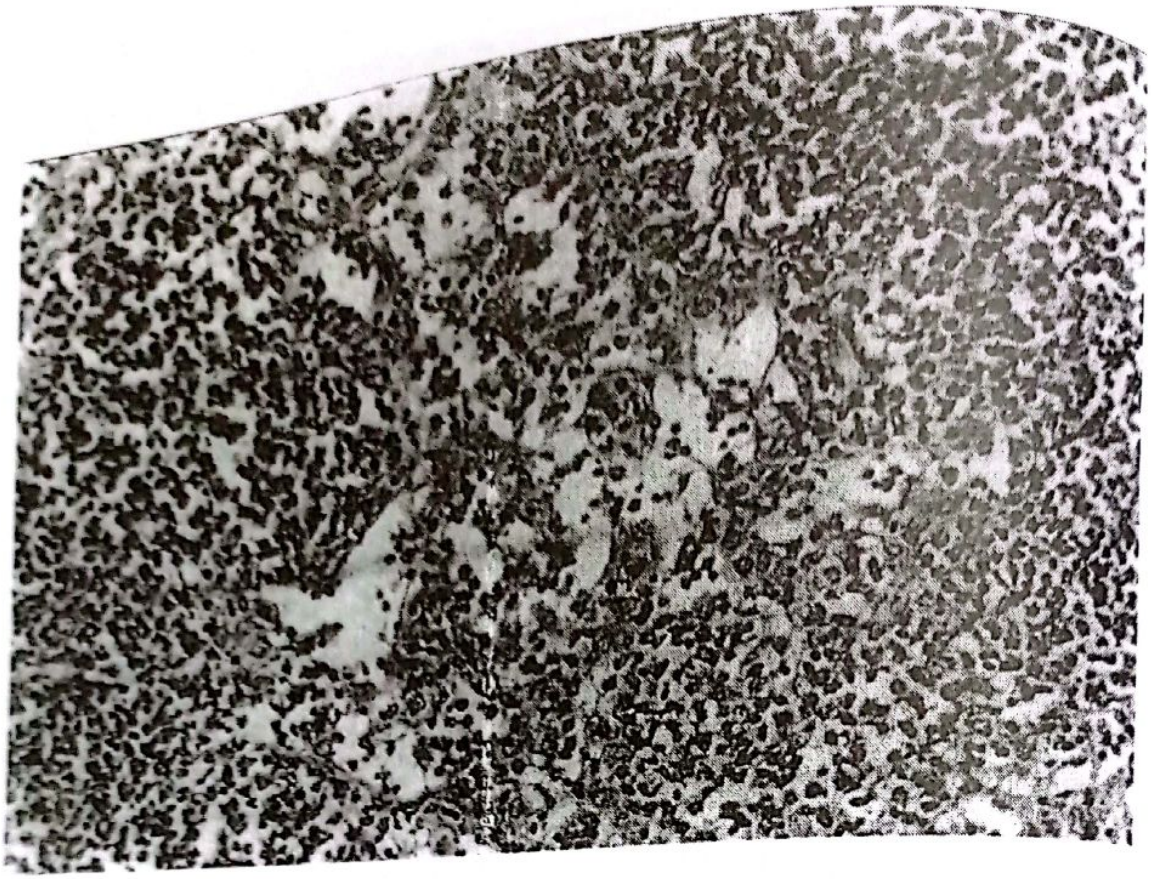

Fig. (7): Thymus of aflatoxicated rabbit showing medullary lymphocytic depletion (H and E x 200).

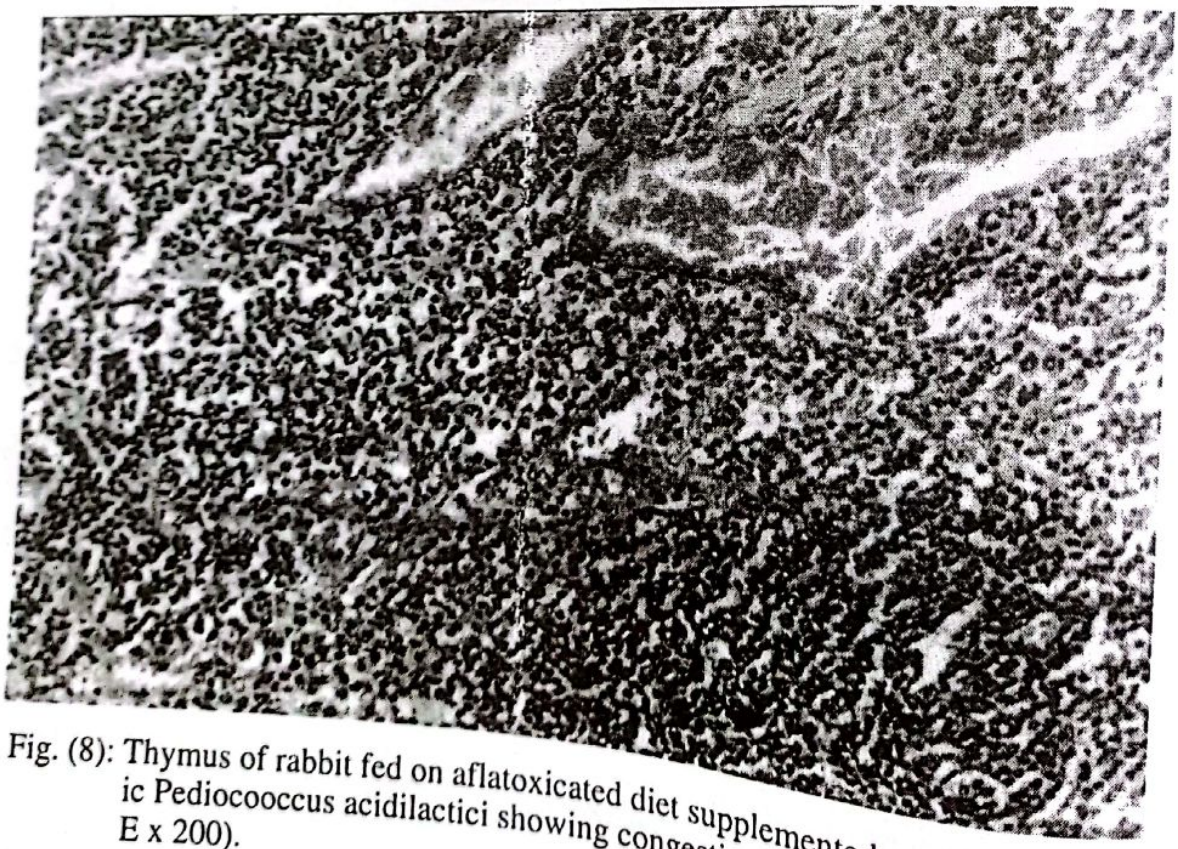

Fig. (8): Thymus of rabbit fed on aflatoxicated diet supplemented with the probiotic Pediocooccus acidilactici showing congestion of blood vessels (H and E x 200).

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تأثير المحفز الحيوى بديوكوكاس اسيدوفلاس على الشذوذات الكروموسومية ، التغيرات الباثولوجيه الأكلينيكيه و المناعية في ذكور الارانب المعرضة للافلا توكسين ب١

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

تم تقسيم الارانب الي اربعة مجموعات،المجموعة الاولي استخدمت كمجموعه ضابطة، المجموعة الثانية تم اعطائها المحفز الحيوي بديوكوكاس اسيدوفلاس (100g/ton diet) لمده 6 اسابيع، ، المجموعة الثالثة تم تعرضها للافلا توكسين ب١ عن طريق خلطها بالعلف (60ug/kg diet) لمده 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) لمده 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 وخاصه خلايا الليمفوسيت و لقد أظهرت التغيرات البيوكيميائيه للدم تحسن ملحوظ حيث انخفض نشاط الألأنين امينو ترانسفيريز و الأسبرتات 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.