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RESEARCH ARTICLE

EFFECTS OF AFLATOXIN B₁ ONLY OR CO-ADMINISTERED WITH MYCOTOX NG ON LIVER FUNCTION IN TURKEY BROILERS

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Abstract

Mycotoxins are mold metabolites of particular significance for human and animal health. Poultry feeds contaminated with aflatoxins incur considerable losses to poultry industry. The aim of the present investigation was to evaluate the effects of aflatoxin B₁ and Mycotox NG applied either independently or together, on blood total protein, albumin, blood glucose, total bilirubin, triglycerides, cholesterol, enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ GT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), and changes in liver morphology. At the same time, the potential of supplementation of feed with a mycosorbent (Mycotox NB) was evaluated. Experiments were carried out with 60 7-day-old female turkey broilers (meat TM strain) divided into one control and five treatment groups (n=10). Groups were as followed: Group I – control (fed standard feed according to the species and age of birds); Group II – experimental, whose feed was supplemented with 0.5 g/kg Mycotox NG, Group III – experimental, whose feed contained 0.2 mg/kg aflatoxin B₁, Group IV – experimental, whose feed contained 0.4 mg/kg aflatoxin B₁, Group V – experimental, supplemented with 0.2 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG and Group VI – experimental, supplemented with 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG. The duration of the experiments was 42 days. The monitored blood chemical parameters were analysed on post treatment days 21 and 42. In birds treated only with AFB₁, increased blood activities of studied enzymes and of total bilirubin was established. At the same time, blood total protein, albumin, cholesterol, glucose and triglycerides were reduced as compared to controls. The observed histopathological changes in the liver consisted in various extent of dystrophy depending on the dose of mycotoxin (congestion, vacuolar and granular dystrophy, round cell proliferation, necrobiotic changes, hyperplasia of gallbladder epithelium). The addition of mycosorbent (Mycotox NG) to the feed of Groups V and VI reduced substantially the changes in blood chemistry and the severity and frequency of liver histological lesions.

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Introduction:-

Liver is the organ responsible for the metabolism and detoxication of a number of xenobiotics in the animal and human body. It performs different functions associated with metabolism, and detoxication of various toxic substances and narcotics (Parsai et al., 2014).

Aflatoxins were first isolated in the 1960s after a lethal outbreak in turkeys (Blount, 1961) and liver cancer in rainbow trouts (Rucker et al., 2002) fed rations containing peanut and cotton meal. Aflatoxins are bioactive substances whose chemical structure differs a little (Cullen and Newberne, 1993), and are produced by molds from the *Aspergillus* genus (*Aspergillus flavus* and *Aspergillus parasiticus*). These fungi are widespread in nutrients as wheat, corn, rice, nuts, peanuts, dried fruits and spices (Rucker et al., 2002). Aflatoxins are involved in the food chain mainly after ingestion through the alimentary tract of men and animals (Aycicek et al., 2005). Aflatoxicosis is a nutritional toxicosis occurring after consumption of foods contaminated with aflatoxins. Two clinical forms of aflatoxicosis are known in animals – acute, manifested with severe liver damage resulting in death and chronic, with symptomatic course (Cullen and Newberne, 1993). Among all identified aflatoxins (AF), AFB₁, AFB₂, AFG₁ and AFG₂ are the four main forms found in naturally contaminated feeds. The toxicity of AFG₂, AFB₂ and AFG₁ is equal to 10%, 20% and 50% of that of AFB₁ respectively (Leeson et al., 1995). AFB₁ is the most toxic and most frequent form found in feed ingredients (Cullen and Newberne, 1993). AFB₁ becomes toxic after metabolic conversion in the liver by microsomal enzymes cytochrome P450 (CYP450) in reactive and electrophilic exo-AFBO (Rawal et al., 2010). Then, it binds to proteins, DNA, RNA and other cellular nucleophils and this, structural and enzymatic functions of protein could be affected causing development of neoplastic processes in the liver parenchyma (Wu and Khlangwiset 2010).

The toxic effects of aflatoxins in domestic poultry are manifested with anorexia, apathy, reduced growth performance, reduced feed intake, increased feed conversion ratio, reduced egg production, altered visceral organs relative weights, increased death rates (Bailey et al., 1998; Kubena et al., 1998; Manafi, 2012; Valchev et al., 2013; Kana et al., 2014); anaemia (Al-Daraji et al., 2004); reduced humoral and cellular immunity (Gabal and Azzam 1998; Oguz et al., 2003); lower blood serum concentrations of total protein, albumin, cholesterol, triglycerides, glucose (Zao et al., 2010; Fani Makki et al., 2014; Kana et al., 2014; Valchev et al., 2014); increased blood total and direct bilirubin (Fani Makki et al., 2014); increased activity of various enzymes – aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (γ GT) and lactate dehydrogenase (LDH) (Kaki et al., 2012; Kalpana et al., 2012; Fani Makki et al., 2014; Kana et al., 2014; Valchev et al., 2014; Azizpour and Moghadam, 2015). Histopathological changes in the liver consisted in hepatomegaly, ochre yellow colour, haemorrhages, vacuolar (fatty) dystrophy of hepatocytes, proliferation of neutrophils and mononuclear cells, proliferation of the biliary duct epithelium, necrotic alterations in the parenchyma, fibrosis (Ekhlās, 2012; Ramdaset al., 2013; Valchev et al., 2014; Azizpour and Moghadam, 2015).

The sensitivity of the different fowl species to the toxic effects of aflatoxins is different. Most sensitive are growing ducklings, goslings, pheasants and broiler chickens (Leeson et al., 1995). From public health point of view, aflatoxins are dangerous for the health of poultry meat consumers as they accumulate and could provoke neoplastic growths (Ishfaq et al., 2014).

Different methods for elimination, inactivation and reduction of bioavailability of aflatoxins in feeds have been implemented: physical (separation, heat, radiation), chemical (ammonia, ozone, sulfites, hypochlorites), biological (bacteria, yeasts) and nutritional (vitamins, minerals). Most of them have at least two disadvantages: high cost of decontamination of feeds and failure to ensure a complete removal of aflatoxins without losing a great part of the nutritional value of feeds (Méndez-Albores *et al.*, 2007; Eshaket al., 2010). One of the most practical methods nowadays is the use of non-nutritional mycosorbents as aluminosilicates, bentonites, clinoptilolites (Oguz et al., 2003; Valchev et al., 2013; Valchev et al., 2014), activated charcoal (Khadem et al., 2012) or bioproducts: yeast cultures (*Saccharomyces cerevisiae*) (Abdel-Wahhab et al., 2002; Valchev et al., 2013; Valchev et al., 2014). Toxin-binders reduce the toxic effects of mycotoxins through binding them and reducing their absorption by the gastrointestinal tract and their bioavailability in blood (Kana et al., 2014).

Material and Methods:-

Experimental design:-

An experiment was performed with 60 7-day-old female turkey broilers (meat TM strain) divided into six groups (n=10).

The experimental design comprised:

Group I – control. Birds were fed standard feed according to the species and age, produced by a feed factory;

Group II – experimental, whose feed was supplemented with 0.5 g/kg Mycotox NG (Ceva Sante Animale, France (micronised yeasts, montmorillonite, thymol));

Group III – experimental, whose feed contained 0.2 mg/kg ϕ ypаж aflatoxin B₁;

Group IV – experimental, whose feed contained 0.4 mg/kg aflatoxin B₁;

Group V – experimental, supplemented with 0.2 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG;

Group VI – experimental, supplemented with 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG. The duration of the experiments was 42 days

Aflatoxin B₁ was produced by *Aspergillus flavus* (99% purity) and obtained from Sigma-Aldrich (Germany). All turkey poultlets were kept under optimum microclimatic parameters according to Ordinance 44/2006.

Blood samples were collected from v. metatarsalis medialis on post treatment days 21 and 42 in sterile heparinised containers (FL medical, Italy) for analysis of enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (γ GT), lactate dehydrogenase (LDH) and blood total protein, albumin, cholesterol, triglycerides, total bilirubin, blood glucose. Samples were centrifuged within 30 min after collection at 1500×g at 4°C for 10 min. Plasma was harvested and stored at -20°C until analysis. The blood chemical parameters were assayed on an automated biochemical analyser BS-120, Mindray, China.

Liver specimens were collected for morphological examination after euthanasia by cervical dislocation as per Ordinance 20 on the minimum requirements for the protection and welfare of experimental animals and requirements to objects for use, cultivation and / or supply (State Gazette 87/9/11/2012). They were fixed in 10% formalin, embedded in paraffin after an ethanol series. Cross sections were stained with haematoxylin/eosin.

The experiments were approved by the Bulgarian Food Safety Agency – permit No 19218/06.11.2014.

Data were statistically processed using single-factor Anova, and the level of statistical significance was determined with the Tukey-Kramer test ($p < 0.05$).

Results:-

Blood chemistry analysis:-

The data from Table 1 showed the effects of AFB₁ or/and Mycotox NG on blood plasma total protein, albumin and glucose on treatment days 21 and 42. By the 21st day, turkeys receiving only AFB₁ (at 0.2 and 0.4 mg/kg feed) had statistically significant lower blood total protein (32.53 ± 0.83 g/l and 28.53 ± 1.19 g/l) and albumin (16.8 ± 0.91 and 15.4 ± 0.60 g/l) respectively ($p < 0.001$) than control birds (46.31 ± 1.46 g/l and 21.6 ± 0.72 g/l). By the 42nd day, the changes were more pronounced with total protein concentrations of 28.01 ± 0.83 g/l and 25.09 ± 1.47 g/l respectively ($p < 0.001$) vs controls (45.59 ± 1.36 g/l) and mean albumin levels in blood of 13.8 ± 0.94 g/l and 12.6 ± 0.52 g/l ($p < 0.001$) respectively as compared to untreated turkeys (21.3 ± 0.61 g/l). In Groups V and VI which were supplemented with 0.2 and 0.4 mg/kg AFB₁ plus 0.5 mg/kg mycosorbent, the values were also reduced by day 21 and day 42 ($p < 0.05$, $p < 0.001$ respectively). Blood total protein levels were 35.89 ± 1.08 g/l and 30.09 ± 0.94 g/l respectively by the 21st day, whereas corresponding albumin concentrations - 19.2 ± 0.77 g/l and 17.7 ± 0.54 g/l. By the 42nd day, the tendency towards lower total protein and albumin levels in blood was preserved as seen from respective total protein (33.74 ± 0.69 g/l; 28.49 ± 1.39 g/l) and albumin concentrations (17.2 ± 0.72 g/l; 16.2 ± 0.80 g/l).

Blood glucose was significantly decreased on the 21st day (12.84 ± 0.57 mmol/l and 12.48 ± 0.39 mmol/l) and 42nd day (10.85 ± 0.33 mmol/l and 9.64 mmol/l) in groups challenged with 0.2 and 0.4 mg/kg AFB₁ compared to controls (15.42 ± 0.48 mmol/l and 15.82 ± 0.46 mmol/l) ($p < 0.01$ - $p < 0.001$). The addition of mycosorbent to the feed of groups V and VI prevented the toxic effects of AFB₁ on blood glucose levels ($p > 0.05$) - 14.15 ± 0.60 mmol/l and 13.52 ± 0.42

mmol/l respectively on day 21; or reducing effect 13.79 ± 0.45 mmol/l and 13.00 ± 0.36 mmol/l respectively on day 42 ($p < 0.01$ - $p < 0.001$).

The changes in total bilirubin (Table 2) demonstrated statistically significantly higher values in turkey broilers treated at increasing doses of AFB₁ (0.2 mg/kg or 0.4 mg/kg) on days 21 and 42. The values on the 21st day were 11.21 ± 0.22 μ mol/l and 12.01 ± 0.37 μ mol/l vs controls (8.31 ± 0.60 μ mol/l, $p < 0.001$), whereas on the 42nd day: 9.86 ± 0.41 μ mol/l and 11.10 ± 0.31 μ mol/l respectively ($p < 0.001$) vs controls (7.52 ± 0.23 μ mol/l). The deleterious effects of AFB₁ on blood bilirubin was attenuated in groups V and VI by the addition of 0.5 g/kg Mycotox NG. The detected values in these groups were 10.21 ± 0.25 μ mol/l and 10.54 ± 0.42 μ mol/l (day 21) and 8.98 ± 0.32 μ mol/l and 9.47 ± 0.38 μ mol/l (day 42) – partly reduced than control values ($p < 0.05$ - $p < 0.01$).

Total cholesterol and triglycerides (Table 2) in blood were substantially reduced at both sampling intervals in groups treated with AFB₁ only (Groups III, IV) ($p < 0.001$). On the 21st day respective cholesterol values were 3.76 ± 0.15 mmol/l and 3.40 ± 0.19 mmol/l vs 4.67 ± 0.12 mmol/l in controls, while those of triglycerides -1.45 ± 0.020 mmol/l and 1.39 ± 0.039 mmol/l ($p < 0.001$ vs controls: 1.79 ± 0.053 mmol/l). These changes tended to become more severe after another 21 days: blood cholesterol became 3.29 ± 0.12 mmol/l and 2.96 ± 0.12 mmol/l respectively ($p < 0.001$ vs controls with 4.68 ± 0.12 mmol/l). Blood triglycerides in groups III and IV attained 1.36 ± 0.043 mmol/l and 1.23 ± 0.032 mmol/l as compared to controls (1.72 ± 0.041 mmol/l; $p < 0.001$). The harmful influence of AFB₁ on blood cholesterol and triglycerides in groups V and VI were partly reduced (3.95 ± 0.13 mmol/l; 3.61 ± 0.12 mmol/l and 1.59 ± 0.031 mmol/l; 1.44 ± 0.032 mmol/l on day 21 and 3.99 ± 0.17 mmol/l; 3.71 ± 0.18 mmol/l and 1.50 ± 0.052 mmol/l; 1.40 ± 0.037 mmol/l on day 42) by addition of 0.5 g/kg Mycotox NG to feed ($p < 0.05$ and $p < 0.001$ respectively).

The changes in plasma aminotransferase activities (AST, ALT and γ GT) as well as activities of LDH and AP are presented in Tables 3 and 4. Blood AST, ALT and γ GT concentrations were statistically significantly higher ($p < 0.001$) in turkey broilers treated with AFB₁ only (Groups III and IV) during both periods (days 21 and 42). On the 21st day, respective levels were 160.0 ± 4.39 U/l and 191.0 ± 4.39 U/l for AST; 28.47 ± 1.06 U/l and 31.29 ± 1.77 U/l for ALT; 38.6 ± 1.49 U/l and 53.2 ± 3.72 U/l for γ GT. On day 42, observed changes were more pronounced: AST concentrations were 298.2 ± 3.74 U/l and 342.1 ± 8.06 U/l; ALT – 39.09 ± 0.62 U/l and 45.06 ± 2.05 U/l; γ GT – 57.4 ± 1.96 U/l and 78.0 ± 3.09 U/l respectively. The supplementation of feed of Groups V and VI with 0.5 g/kg Mycotox NG reduced considerably ($p < 0.05$, $p < 0.001$) the adverse effects of the mycotoxin on blood aminotransferases. On the 21st day, AST, ALT and γ GT values in birds from Group V were respectively – 143.8 ± 3.42 U/l, 21.41 ± 0.35 U/l and 32.00 ± 3.04 U/l, while in Group VI – 169.5 ± 3.79 U/l, 24.34 ± 0.99 U/l and 40.7 ± 1.57 U/l. By the 42nd day, the tendency was preserved: AST levels attained 247.4 ± 4.17 U/l (Group V) and 268.8 ± 4.00 U/l (Group VI); ALT – 30.73 ± 0.74 U/l and 37.3 ± 1.45 respectively; while γ GT was 42.7 ± 2.05 U/l in Group V and 61.4 ± 1.55 in Group VI.

The changes in blood LDH and AP in groups III and IV were similar. By the 21st day, LDH activity was 479.2 ± 7.52 U/l in Group III and 535.2 ± 13.50 U/l in Group IV ($p < 0.001$) vs controls (427.3 ± 3.90 U/l) and of AP: 85.1 ± 1.66 U/l (Group III), 103.3 ± 5.07 U/l (Group IV) and 57.4 ± 3.29 U/l ($p < 0.001$) (control group). By the 42nd day observed changes in LDH and AP were more obvious as compared to control birds ($p < 0.001$). LDH became 541.5 ± 6.28 U/l in Group III and 588.4 ± 8.02 U/l in Group IV vs 439.4 ± 3.57 U/l in controls. AP activity increased up to 139.5 ± 7.52 U/l in Group III and 160.7 ± 6.92 U/l in Group IV as compared to untreated birds (61.3 ± 2.01 U/l). In Groups V and VI, the deleterious effects of AFB₁ on LDH and AP were partly neutralised ($p < 0.01$ - $p < 0.001$) with LDH concentrations on day 21 of 476.6 ± 8.40 U/l and 509.6 ± 3.63 U/l respectively. By the 42nd day, LDH was 521.8 ± 4.56 U/l (Group V) and 533.3 ± 9.37 U/l (Group VI). Alkaline phosphatase values were 75.4 ± 1.78 U/l and 88.6 ± 2.50 U/l (day 21) vs 106.2 ± 5.22 U/l and 128.9 ± 2.36 U/l (day 42).

Histopathological studies:-

Birds fed the standard feed only (control group) and receiving only Mycotox NG (Group II) did not exhibit gross changes in normal liver architectonics.

Turkey poults treated with 0.2 mg/kg AFB₁, demonstrated congestion in their livers – strongly dilation of capillaries with activation of the endothelium, pericapillary oedema, granular degradation of the cytoplasm of hepatocytes (Fig. 1). In some areas, round cell proliferations were visible (Fig. 2).

The birds treated at 0.4 mg/kg AFB₁ showed strongly impaired liver structure. Apart the congestive events in blood vessels and round cell proliferations, necrobiotic areas and vacuolar degeneration in many hepatocytes were present (Fig. 3). A part of the biliary duct epithelium was hyperplastic (Fig. 4).

Turkeys treated with 0.2 mg/kg AFB₁ and 0.5 g/kg Mycotox NG showed considerably milder liver dystrophic changes than birds treated with 0.2 mg/kg AFB₁ only: generalised vascular hyperaemia, milder vacuolar degeneration of hepatocytes (Fig. 5). Single round cell proliferations were also occasionally seen (Fig. 6).

Turkeys treated at 0.4 mg/kg ϕ ypаж AFB₁ and 0.5 g/kg ϕ ypаж Mycotox NG exhibited widened sinusoidal spaces and reduced size of hepatocytes (Fig. 7). At the same time, fibrous connective tissue proliferation was observed in perilobular spaces (Fig. 8).

Discussion:-

Fungi alter the colour of cereal crops, their nutritional and chemical characteristics, reduce seed germination and contaminate the feed with mycotoxins, which are highly toxic for men and animals. *Aspergillus flavus* and *Aspergillus parasiticus* are among the most important fungal producers of aflatoxins in storehouses, commonly present in cereals and cereal animal feeds (Paster et al., 1993).

Aflatoxins are of particular importance for the poultry industry due to their high toxicity and frequent contamination of feeds. Feeds contaminated with aflatoxins reduce the activity of numerous enzymes involved in the metabolism of carbohydrates, proteins, fats and nucleic acids (Azizpour and Moghadam, 2015). Chronic and subchronic aflatoxicosis could be detected through analysis of changes in blood serum biochemical and haematological parameters before any clinical signs become visible. The turkeys are especially susceptible to toxic effects of AFB₁. The liver glutathione S-transferases alpha class (GSTA) in this species is not able to detoxify AFB₁, which is probably the main factor for their high sensitivity (Rawal et al., 2010).

Hepatotoxic effects of AFB₁ were reported in various animal species: ducklings (Cheng et al., 2001); broiler chickens (Ekhtas, 2012; Kana et al., 2014; Valcev et al., 2014); quails (Eraslan et al., 2004); rats (Liu et al., 2001); pigs (Shi et al., 2005); rabbits (Hanafi et al., 2010). Increased blood serum activity of liver enzymes ALT, AST, LDH, ALP, γ GT are indicative for impaired liver function (Denli et al., 2009; Pappa and Padmalatha, 2014; Valcev et al., 2014). Increased activity of transaminases (AST, γ GT and ALT) points at their release from hepatocytes consequently to their damage (increased permeability of cell membranes, cellular necrosis and autolytic degradation of hepatocytes) (Kalpana et al., 2012). Aminotransferases are a class of liver enzymes with important role in the metabolism of amino acids and carbohydrates, involved also in the citric acid cycle through intermediate metabolites. Alkaline phosphatase is a membrane-bound enzyme and the change in its activity as a result of the toxic effects of aflatoxins indicates increased membrane permeability and impaired transport of metabolites (Pappa and Padmalatha, 2014). Increased activity of this enzyme suggests obstructive and degenerative changes in the liver, established in rabbits fed aflatoxin-contaminated diets (Nowar et al., 2001). High levels of ALT are found in the cytoplasm of hepatocytes, and of AST – in mitochondria (Wells, 1988). Increased plasma AST and ALT is a sign of cellular leakage and impaired integrity of liver cell membranes (Parsai et al., 2014). Increased γ -GT activity indicates cholestasis or biliary duct hyperplasia in birds (Mohamed and Mohamed, 2009; Valchev et al., 2014).

Aflatoxins impair the protein synthesis by forming adducts with DNA, RNA and proteins (Busby and Wogan, 1984), RNA synthesis inhibition, reduction of DNA-dependent RNA polymerase activity, degranulation of granulated endoplasmic reticulum (Verma and Nair, 2001), all of them mechanisms through which the structure of various tissues (liver, kidneys, skeletal muscles, heart, pancreas) is altered (Sharma et al., 2011). On the other hand, reduced blood total protein concentrations are probably a result of liver dystrophy and hence, reduced rate of protein synthesis. The lower protein biosynthesis and dystrophic changes of liver cause reduction in plasma protein concentrations observed in this study. The enhanced elimination of proteins through the kidneys is another cause for low plasma values (Shahzad et al., 2014). Hypoproteinaemia can probably be a result of reduced food intake (El-Shewy and Ebrahim, 2004). Lower blood levels of total protein and albumin observed in the present experiments agree with the results of others (Shi et al., 2009; Valchev et al., 2014). Disturbed protein synthesis is a cause for lower concentrations of proteins in skeletal muscle (Verma & Chaudhari, 1999), the heart, liver, and kidneys (Verma and Kolhe, 1997) in animals fed aflatoxin-contaminated feeds.

Bilirubin is a secondary product of haemoglobin conversion, filtered through kidneys and then, conjugated to glucuronic acid in the liver and excreted with the bile. Increased total bilirubin results from impaired liver function in aflatoxicosis (Rustemeyer et al., 2010). Hyperbilirubinaemia is a very sensitive test for detection of the functional integrity of the liver, as well as for assessment of dystrophy severity consequently to aflatoxicosis (Rizvi et al., 2000; Banu et al., 2010).

The present study established lower plasma cholesterol and triglycerides in turkey broilers treated with AFB₁. They result from aflatoxin-impaired lipid metabolism in the liver (Shi et al., 2009).

Lower blood glucose in the present experiments was due to lower intake of food and/or reduced activity of enzymes involved in carbohydrate metabolism and glycogenolysis (glucose-6-phosphatase and fructose-1,6-bisphosphatase) as a result of liver dystrophy or overproduction of insulin (Soliman et al., 2008; Zao et al., 2010; Valchev et al., 2014). Aflatoxins inhibit the protein synthesis and thus, the excess of amino acids could be used to meet the energy needs of the body and maintenance of blood glucose levels, but impaired gluconeogenesis could cause lower blood glucose levels (Soliman et al., 2008). Hypoglycaemia could probably result from enhanced glycolysis or reduced absorption of glucose through the intestines (Semenov et al., 2016).

Our observations on liver morphology changes are in line with data reported by Espada et al., (1992); Oguz et al., (2000); Kumar and Balachandran (2009); Zao et al., (2010); Ekhlas, (2012). Liver dystrophy was due to damage of main cell macromolecules (lipids, proteins, DNA). They result from oxidative stress induced by aflatoxins, a mechanism provoking oxidative damage in DNA and lipid peroxidation (Mohamed and Mohamed, 2009). On the other hand, the accumulation of calcium in hepatocytes provokes mitochondrial dysfunction and reduced rate of adenosine triphosphate synthesis, resulting in morphological disturbances of liver structure (Quezada et al., 2002; Fatemi et al., 2006). The liver is a target organ for the toxic effect of AFB₁, as in the liver aflatoxins undergo bioactivation to reactive 8,9-epoxide, which binds to DNA and proteins. As a result, the normal liver structure is damaged (Pasha et al., 2007). Aflatoxin B₁ is cytotoxic for hepatocytes and inhibits their proliferation (Abdel-Wahhab et al., 2007). The observed histopathological changes in the liver parenchyma are due to the accumulation of fats in hepatocytes following impaired lipid metabolism, which in turn is caused by inhibited synthesis of phospholipids and cholesterol causing impairment of liver lipid transport (Espada et al., 1992).

Bile duct epithelium hyperplasia results from the direct toxic effects of AFB₁ on biliary epithelium or from overproduction of prostaglandins following AFB₁-induced lipid peroxidation (Quist et al., 2000; Saif et al., 2003; Mohamed and Mohamed, 2009).

Compared to control group of birds, the addition of 0.5 g/kg Mycotox NG to the feed of Groups V and VI reduced partly the deleterious effects of aflatoxin B₁ on blood concentrations of studied biochemical blood parameters and the extent of histological liver changes. It is presumed that most of aflatoxin molecules are absorbed by feed ingredients in the gastrointestinal tract causing less adverse effects on the histostucture of target organs and blood biochemical parameters (Azizpour and Moghadam, 2015). These data are in concordance with those reported by other authors having used brewers' yeasts (*Saccharomyces cerevisiae*) (Che et al., 2011; Valchev et al., 2014) and bentonite (montmorillonite) (Shi et al., 2009; Valchev et al., 2014). The process of binding of aflatoxins with toxin-binders is based on the principle of electric polarity. The negative polarity of mycotoxins is bound by the positive polarity of the toxin binder, so the toxins are immobilised and eliminated from the animal body (Kana et al., 2014).

Conclusion:-

The results of the present study showed that the supplementation of turkey broiler feed with AFB₁ had a negative effect on liver function as seen from the increased blood enzyme activities of AST, ALT, γ GT, LDH and AP and of total bilirubin. At the same time, the tested doses of aflatoxin B₁ provoked reduction of plasma total protein, albumin, blood glucose, triglycerides and cholesterol. Increased doses of AFB₁ (0.2 or 0.4 mg/kg feed) induced specific histological changes in the liver. In this study, the addition of 0.5 g/kg Mycotox NG to poultry diets contaminated with 0.2 or 0.4 mg/kg AFB₁, was capable to alleviate the severity of changes in analysed parameters and to reduce the severity of histological lesions induced by aflatoxicosis.

Table 1:- Effects of aflatoxin B₁ (AFB₁) applied independently or combined with Mycotox NG on blood plasma total protein, albumin and glucose in turkey broilers

Groups	Total protein (g/l)		Albumin (g/l)		Glucose(mmol/l)	
	day 21	day 42	day 21	day 42	day 21	day 42
I	46.31± 1.46	45.59± 1.36	21.6± 0.72	21.3± 0.61	15.42± 0.48	15.82± 0.46
II	45.58± 0.93	46.22± 1.44	22.00± 1.05	21.6± 0.87	15.12± 0.43	16.34± 0.44
III	32.53± 0.83 ^{1c,2c}	28.01± 0.83 ^{1c,2c}	16.8± 0.91 ^{1c,2c}	13.8± 0.94 ^{1c,2c}	12.84± 0.57 ^{1b,2a}	10.85± 0.33 ^{1c,2c}
IV	28.53± 1.19 ^{1c,2c,,}	25.09± 1.47 ^{1c,2c}	15.4± 0.60 ^{1c,2c}	12.6± 0.52 ^{1c,2c}	12.48± 0.39 ^{1c,2b}	9.64± 0.64 ^{1c,2c}
V	35.89± 1.08 ^{1c,2c,4c}	33.74± 0.69 ^{1c,2c,3a,4c}	19.2± 0.77 ^{1a,2a}	17.2± 0.72 ^{1c,2b,3a}	14.15± 0.60	13.79± 0.45 ^{2b,3c,4c}
VI	30.09± 0.94 ^{1c,2c,5a}	28.49± 1.39 ^{1c,5a}	17.7± 0.54 ^{1a,2a,}	16.2± 0.80 ^{1c,2c}	13.52± 0.42	13.00± 0.36 ^{2c,3b,4c}

Data are presented as mean ± SEM; n=10 turkey broilers per group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 – vs experimental group I; 3 – vs experimental group II; 4– vs experimental group I1; 5– vs experimental group IV

Table 2:- Effects of aflatoxin B₁ (AFB₁) applied independently or combined with Mycotox NG on blood plasma total bilirubin, cholesterol and triglycerides in turkey broilers.

Groups	Total bilirubin µmol/l		Cholesterol mmol/l		Triglycerides mmol/l	
	day 21	day 42	day 21	day 42	day 21	day 42
I	8.31± 0.60	7.52± 0.23	4.67± 0.12	4.68± 0.11	1.79± 0.053	1.72± 0.041
II	8.28± 0.63	7.37± 0.26	4.74± 0.13	4.67± 0.15	1.74± 0.034	1.73± 0.052
III	11.21± 0.22 ^{1c,2c}	9.86± 0.41 ^{1c,2c}	3.76± 0.15 ^{1c,2c}	3.29± 0.12 ^{1c,2c}	1.45± 0.020 ^{1c,2c}	1.36± 0.043 ^{1c,2c}
I V	12.01± 0.37 ^{1c,2c}	11.10± 0.31 ^{1c,2c,3b}	3.40± 0.19 ^{1c,2c}	2.96± 0.12 ^{1c,2c}	1.39± 0.039 ^{1c,2c}	1.23± 0.032 ^{1c,2c}
V	10.21± 0.25 ^{1a,2a}	8.98± 0.32 ^{1a,2a,4c}	3.95± 0.13 ^{1a,2b}	3.99± 0.17 ^{1a,2a,3a,4c}	1.59± 0.031 ^{1a,2a,4b}	1.50± 0.052 ^{1a,2b,4c}
V I	10.54± 0.42 ^{1a,2b}	9.47± 0.38 ^{1b,1b,4b}	3.61± 0.12 ^{1c,2c}	3.71± 0.18 ^{1c,2c,4b}	1.44± 0.032 ^{1c,2c,}	1.40± 0.037 ^{1c,2c}

Data are presented as mean ± SEM; n=10 turkey broilers per group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 – vs experimental group I; 3 – vs experimental group II; 4– vs experimental group I1; 5– vs experimental group IV

Table 3:- Effects of aflatoxin B₁ (AFB₁) applied independently or combined with Mycotox NG on blood plasma AST, ALT and γ -glutamyl transferase activities in turkey broilers.

Groups	AST (U/L)		ALT (U/L)		γ GT (U/L)	
	day 21	day 42	day 21	day 42	day 21	day 42
I	124.4± 2.45	130.9± 1.52	16.53± 0.49	18.25± 0.39	21.8± 0.74	23.8± 10.3
II	124.0± 2.17	134.4± 1.44	16.8± 0.39	18.71± 0.45	22.4± 0.69	24.1± 1.11
III	160.0± 4.39 ^{1c,2c}	298.2± 3.74 ^{1c,2c}	28.47± 1.06 ^{1c,2c}	39.09± 0.62 ^{1c,2c}	38.6± 1.49 ^{1c,2c}	57.4± 1.96 ^{1c,2c}
IV	191.0± 4.39 ^{1c,2c,3a}	342.1± 8.06 ^{1c,2c,3c}	31.29± 1.77 ^{1c,2c}	45.06± 2.05 ^{1c,2c,3b}	53.2± 3.72 ^{1c,2c,3c}	78.0± 3.09 ^{1c,2c,3c}
V	143.8± 3.42 ^{1a,2b,3a,4c}	247.4± 4.17 ^{1c,2c,3c,4c}	21.41± 0.35 ^{1a,2a,3c,4c}	30.73± 0.74 ^{1c,2c,3b,4c}	32.00± 3.04 ^{1a,2a,4c}	42.7± 2.05 ^{1c,2a,3c,4c}
VI	169.5± 3.79 ^{1c,2c,4b,5c}	268.8± 4.00 ^{1c,2c,3c,4c,5a}	24.34± 0.99 ^{1c,2c,4c}	37.3± 1.45 ^{1c,2c,4b,5a}	40.7± 1.53 ^{1c,2c,4b}	61.4± 1.55 ^{1c,2c,3c,4c}

Data are presented as mean \pm SEM; n=10 turkey broilers per group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 – vs experimental group I; 3 – vs experimental group II; 4 – vs experimental group I1; 5 – vs experimental group IV

Table 4:- Effects of aflatoxin B₁ (AFB₁) applied independently or combined with Mycotox NG on blood plasma LDH and AP activities in turkey broilers.

Groups	LDH (U/L)		AP (U/L)	
	day 21	day 42	day 21	day 42
I	427.3± 3.90	439.4± 3.57	57.4± 3.29	61.3± 2.01
II	433.6± 3.63	441.2± 5.38	61.5± 2.14	58.3± 2.40
III	479.2± 7.52 ^{1c,2c}	541.5± 6.28 ^{1c,2c}	85.1± 1.66 ^{1c,2c}	139.5± 7.52 ^{1c,2c}
IV	535.2± 13.50 ^{1c,2c,3a}	588.4± 8.02 ^{1c,2c,3c}	103.3± 5.07 ^{1c,2c,3b}	160.7± 6.92 ^{1c,2c,3c}
V	476.6± 8.40 ^{1b,2a,4c}	521.8± 4.56 ^{1c,2c,4c}	75.4± 1.78 ^{1b,2a,4c}	106.2± 5.22 ^{1c,2c,4c}
VI	509.6± 3.63 ^{1c,2c,4c}	533.3± 9.37 ^{1c,2c,4b,5a}	88.6± 2.50 ^{1c,2c,4a,5a}	128.9± 2.36 ^{1c,2c,4c,5a}

Data are presented as mean \pm SEM; n=10 turkey broilers per group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 – vs experimental group I; 3 – vs experimental group II; 4 – vs experimental group I1; 5 – vs experimental group IV

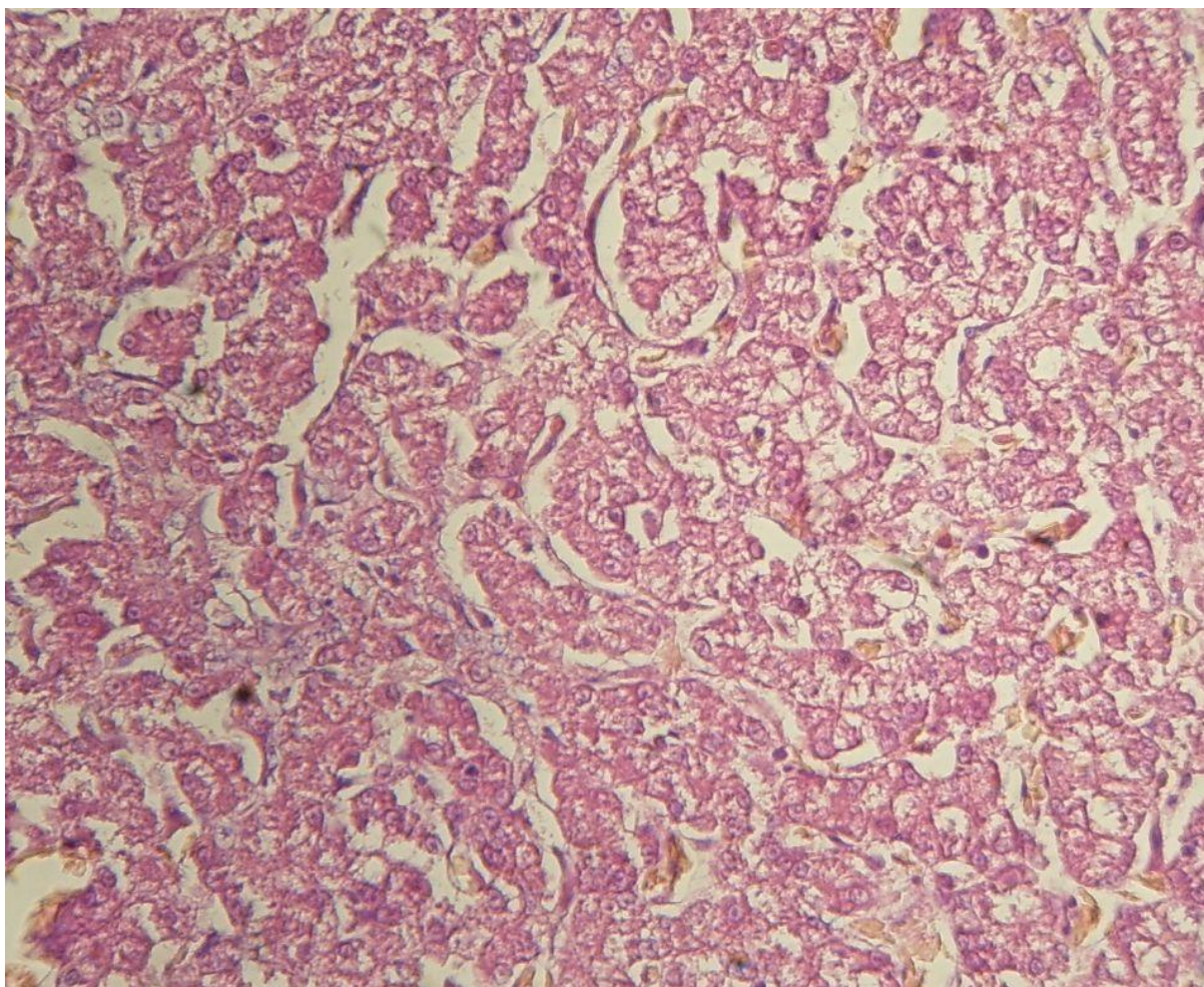


Fig. 1:- Hyperaemia of sinusoidal capillaries with activation of their endothelium and granular destruction of hepatocytes' cytoplasm in turkey broilers treated with 0.2 mg/kg AFB₁. H/E. Bar 15 μ m.

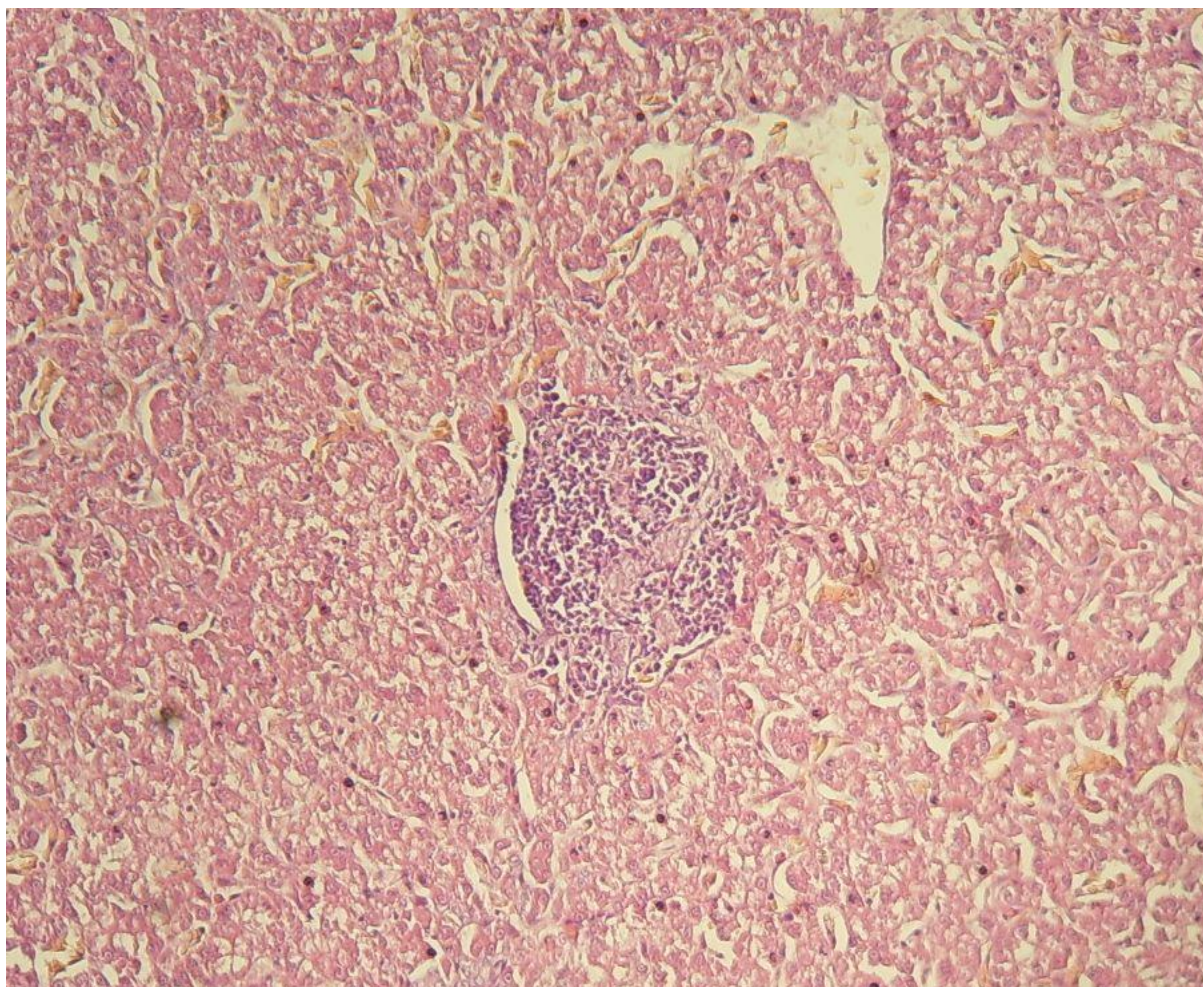


Fig. 2:- Nonnuclear cell proliferation and severe dystrophic changes of hepatocytes in turkey broilers treated with 0.2 mg/kg AFB₁. H/E. Bar20 \square m.

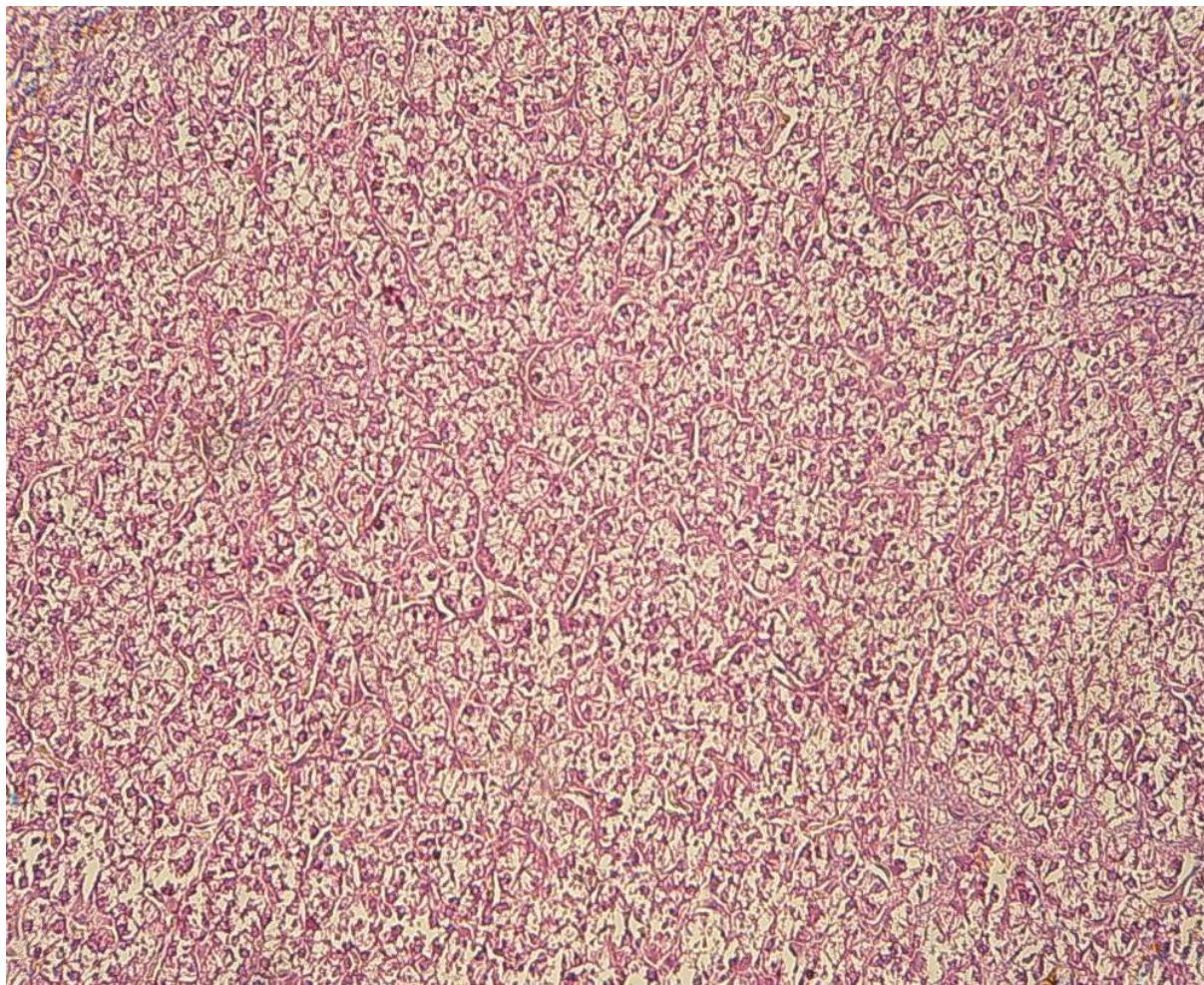


Fig. 3:- Severe degenerative-necrotic processes in hepatocytes with lysis and pyknosis of cell nuclei in turkey broilers treated with 0.4 mg/kg AFB₁. H/E. Bar20 \square m.

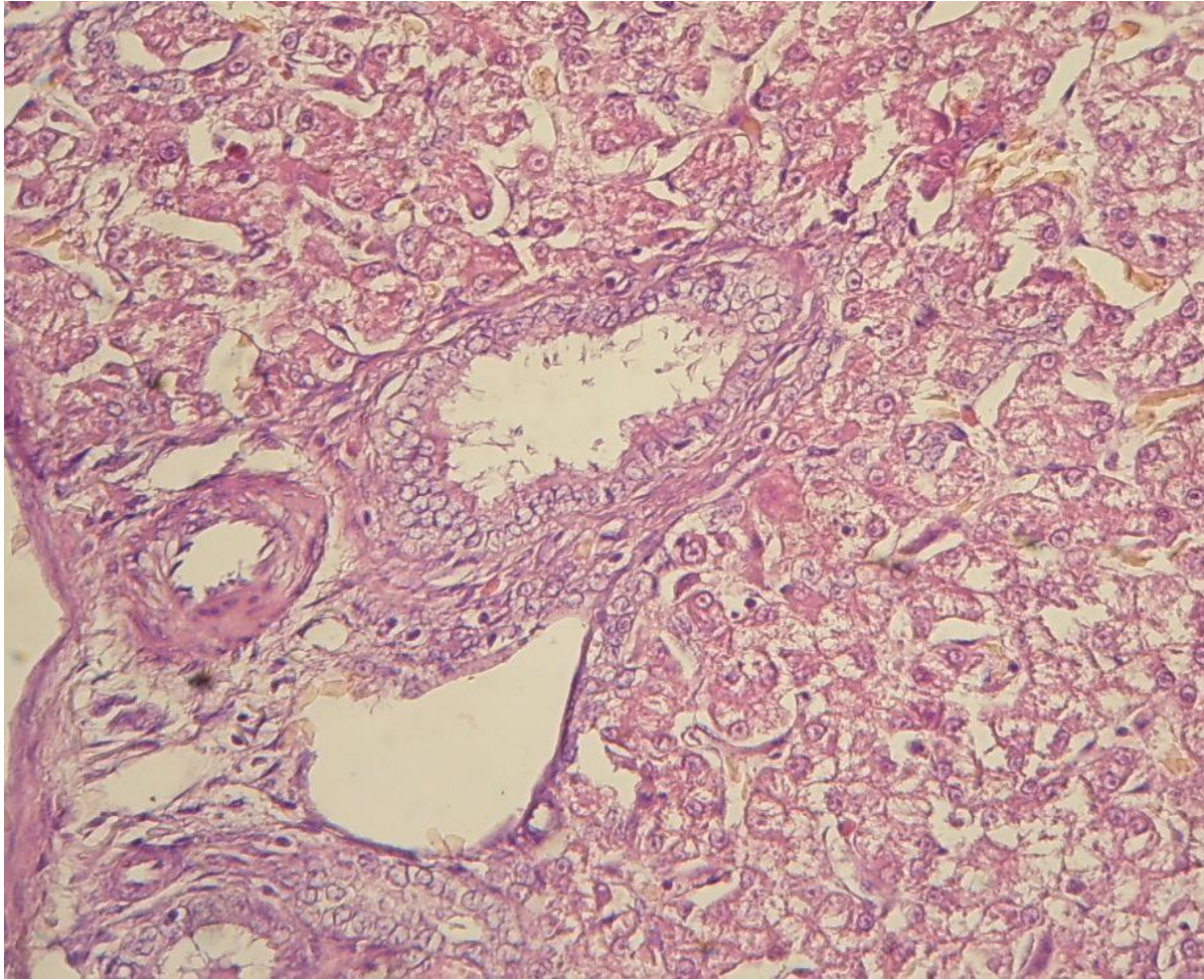


Fig. 4:- Biliary duct hyperplasia in turkey broilers treated with 0.4 mg/kg AFB₁. H/E. Bar15 μ m.

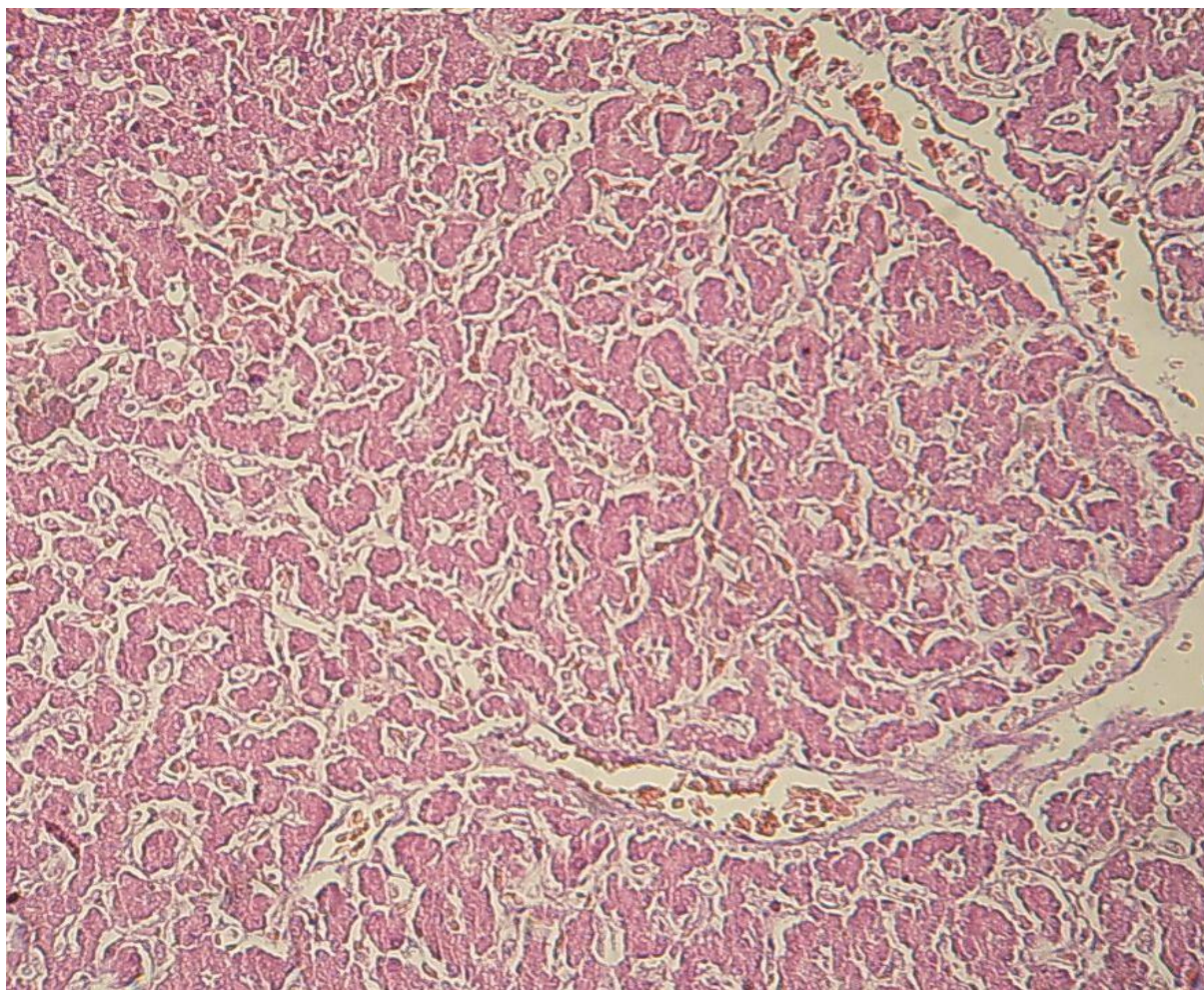


Fig. 5:- Generalised vascular hyperaemia and milder vacuolar degeneration of hepatocytes in turkey broilers treated with 0.2 mg/kg AFB₁ and 0.5 g/kg Mycotox NG. H/E. Bar15 \square m.

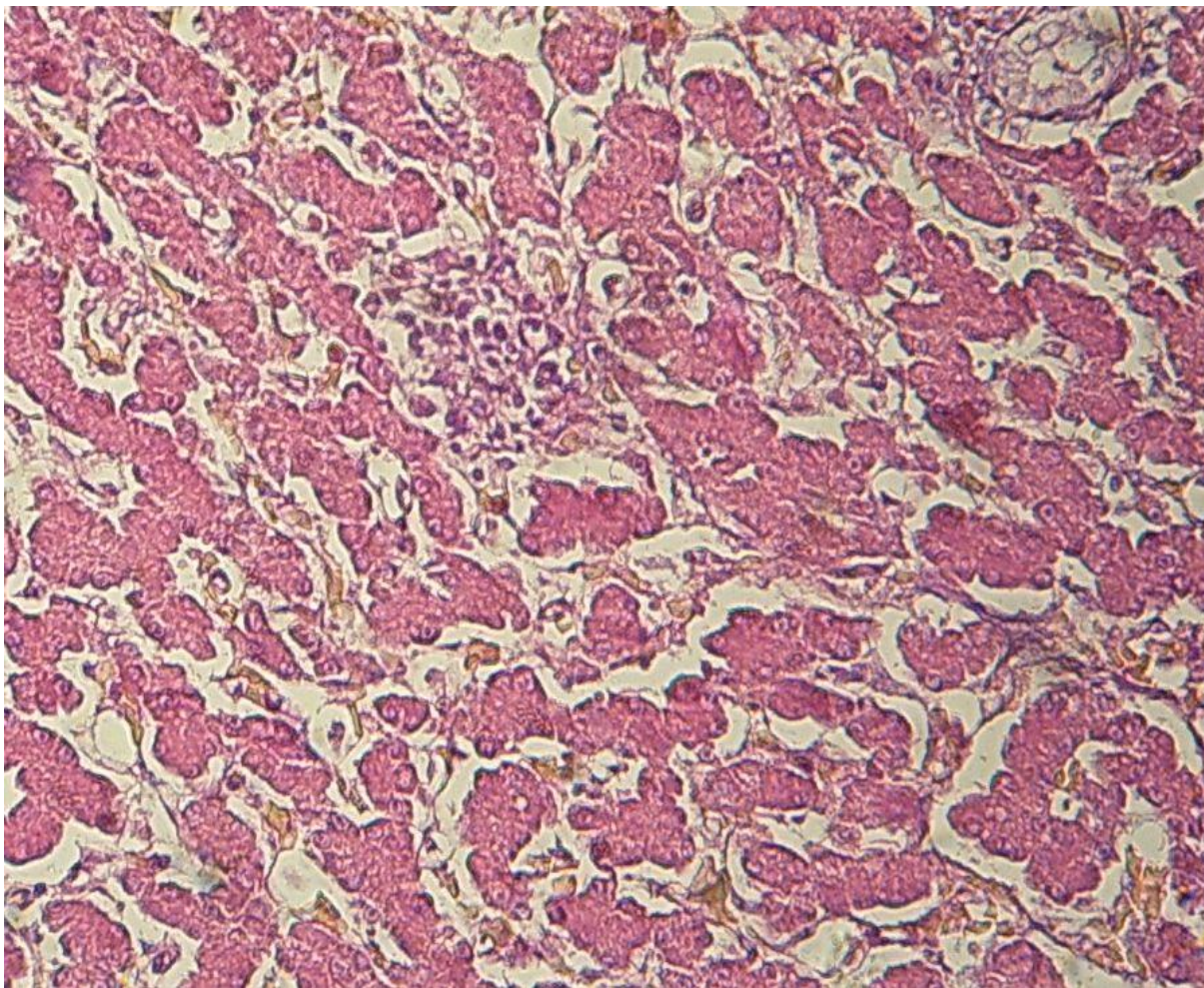


Fig. 6:- Mononuclear proliferations among the liver parenchyma in turkey broilers treated with 0.2 mg/kg AFB₁ and 0.5 g/kg Mycotox NG. H/E. Bar 15 μ m.

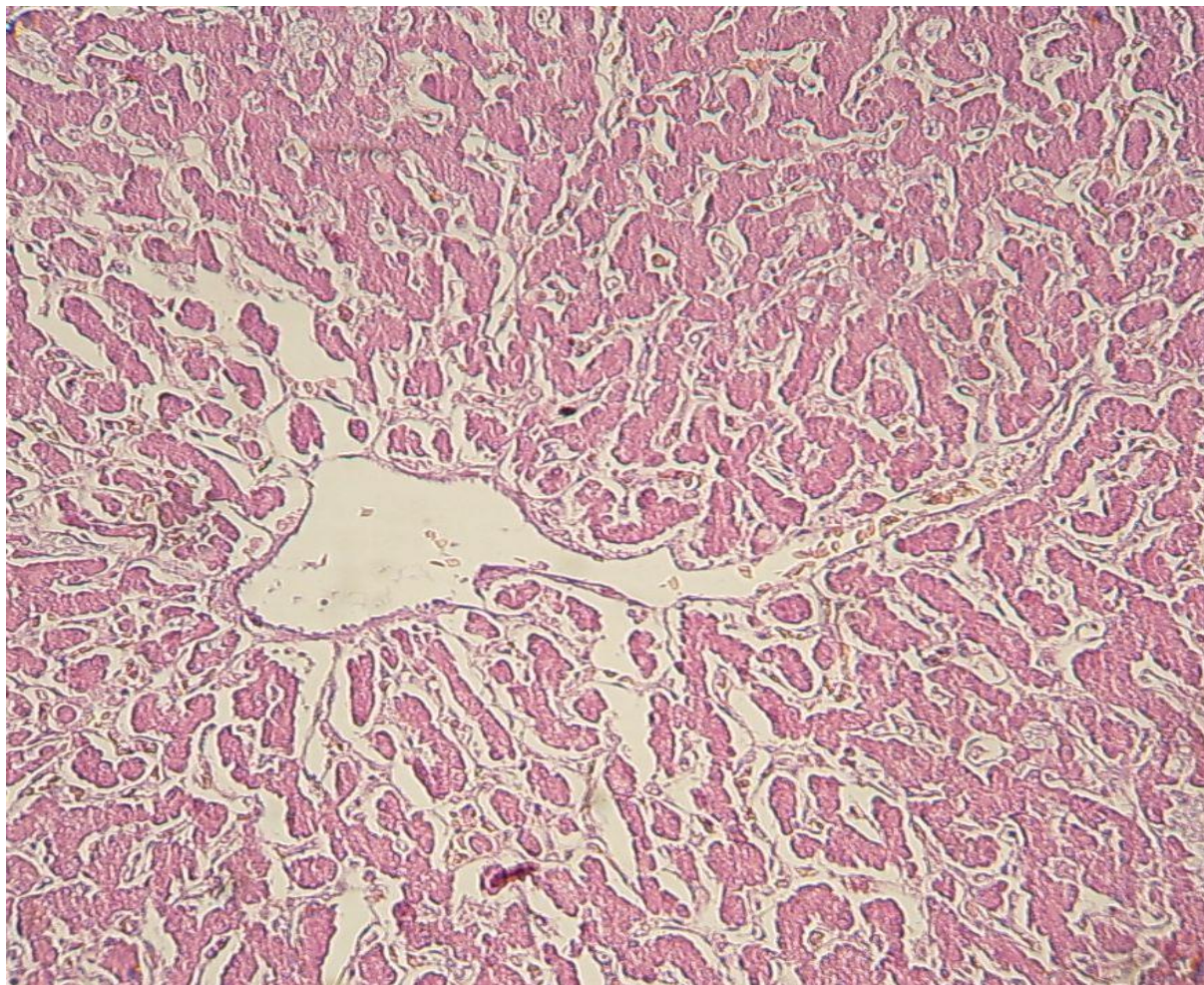


Fig. 7:- Widening of sinusoidal spaces and reduced size of hepatocytes in turkey broilers treated with 0.4 mg/kg AFB₁ and 0.5 g/kg Mycotox NG. H/E. Bar15 □ m.

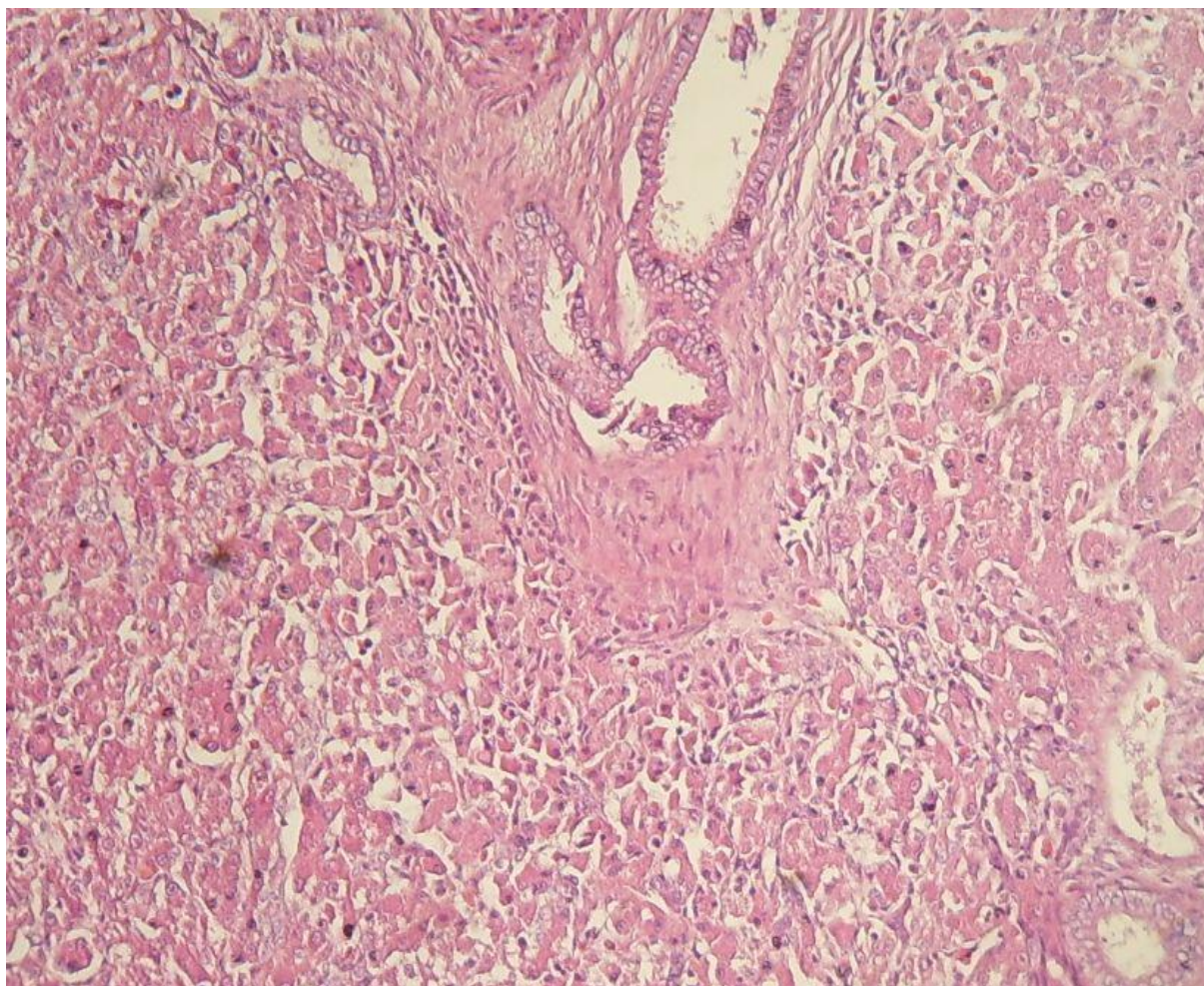


Fig. 8:- Proliferation of fibrous connective tissue in the perilobular spaces in turkey broilers treated with 0.4 mg/kg AFB₁ and 0.5 g/kg Mycotoxin NG. H/E. Bar 15 μ m.

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