Original Article

Study of Protective Effect of Date and Nigella Sativa on Aflatoxin B_1 Toxicity

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Abstract

Background: Many medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. Seeds of *Nigella sativa*, a dicotyledon of the Ranunculaceae family, have been utilized for thousands of years as a spice and food preservative.

Methods: the toxic effect of aflatoxin-B_1 (AFB_1) and the possible cytoprotective effect of *Nigella sativa* (NS) oil and aqueous extract of date were studied on 40 male rats. The animals were divided into 4 groups (10 rats each) and treated daily for two weeks. Group 1 received normal saline as controls. Group 2 treated via intraperitoneal (IP) route with AFB_1 (50µg/kg BW). Group 3 treated with AFB_1 and NS oil via IP. Group 4 treated with AFB_1 and received orally aqueous extract of date (15mg/15ml). The liver and kidneys of each animal were histological examined and biochemical evaluation of the liver and kidney functions was performed.

Results: Group 2 showed severe degenerative and necrotic changes in the liver and kidney. The plasma levels of alanine transaminase (ALT), aspartate transaminase (AST), creatinine and urea in AFB_1 group were significantly higher than the control group. Livers and kidneys of rats, treated with AFB_1 and NS showed less histopathological changes in comparison with the AFB_1 treated group. Livers and kidneys of rats treated with AFB1 and date group showed only mild histopathological changes in comparison with AFB_1 treated group. These histopathological changes seen in animals treated with AFB1 and dates were associated with a significant reduction in levels of ALT, AST, creatinine and urea. Likewise, histopathological changes in the AFB1 and NS group were associated with significant reduction in the levels of beforementioned indices. Moreover, AFB1 and date group showed significant improvement in liver function comparing with AFB_1 and NS group.

Conclusion: our study revealed that treatment with AFB_1 induced histopathological changes in the tissues of liver and kidney associated with dysfunction of these organs. Both NS and date reduce the toxic effects of AFB_1 in liver and kidney. But date treatment was more cytoprotective for liver than NS treatment against aflatoxicosis in rats.

Keywords: *Nigella sativa*; Date, Aflatoxin B1 toxicity; Liver, Kidney, rats.

Introduction

Mycotoxins, are structurally diverse toxic fungal metabolites. They represent the most important category of biologically produced natural toxins relative to human health and economic impact worldwide. [1, 2] Spurred by the discovery of aflatoxin in the 1960s

the first cases of mycotoxicosis were noted leading to the identification of more than 100 toxigenic fungi and in excess of 300 mycotoxins worldwide. [3,4] The diverse chemical structures of mycotoxins account for their differing biological properties and effects. Depending upon the toxins' precise biochemical nature, it may have any of a number of toxic properties including being carcinogenic, tetratogenic, mutagenic, oestrogenic, neurotoxic, or immunotoxic.

Aflatoxins represent a group of closely related difuranocoumarin compounds produced as secondary fungal metabolites of the common molds *Aspergillus flavus, Aspergillus parasiticus* and to a lesser extent *Aspergillus nominus*. There are three strains of Aspergillus from which four major aflatoxins B_1, B_2, G_1, and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) are produced. Among these aflatoxins, AFB_1 is the most prevalent and toxic with acute toxicity demonstrated in all species of animals. AFB_1 is also known as being one of the most potent genotoxic agents and hepatocarcinogens. [4,5]

The toxicity and carcinogenicity of AFB_1 is thought to be directly linked to its bioactivation, that results in formation of a highly reactive AFB_1 8,9-epoxide. The bioactivation of AFB_1 occurs primarily by a microsomal cytochrome P450. It is dependent on epoxidation of the terminal furan ring of AFB_1 and is responsible for binding to cellular macromolecules such as DNA, RNA and other protein constituents. [6,7] Damage of hepatocytes and the kidney is believed to be the result of this process. [8]

Interest in medicinal plants has burgeoned by the increased efficiency of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The use of plants as medicines dates to the down of history.[9, 10] Medicinal plants serve as therapeutic alternatives, safer choices, or in some cases, as the only effective treatment. People in separate cultures and places are known to have used the same plants for similar medical problems. A larger number of these plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects. [10-13]

Among the promising medicinal plants, N. sativa, a dicotyledon of the Ranunculaceae family, is an amazing herb with a rich historical and religious background. [14] The seeds of N. sativa are the source of the active ingredients of this plant. It is the black seed referred to by the Prophet Mohammed (pbuhs) as having healing powers. [14] Black seed is also identified as the curative black cumin in the Holy Bible and is described as the Melanthion of Hippocrates and Discroides and as the Gith of Pliny. [15]

Historically, it has been recorded that N. sativa seeds were prescribed by ancient Egyptian and Greek physicians to treat headache, nasal congestion, toothache, and intestinal worms. It was used as a diuretic, and to promote menstruation and increase milk production.[14] The seeds of N. sativa, known as black seed, black cumin or "Habatul-Barakah," have long been used in folk medicine in the Middle and Far East as a traditional medicine for a wide range of illnesses, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems.[16] Its use in some skin conditions such as eczema has also been recognized worldwide.[14] The seeds can be ground to a powder, mixed with a little flour as a binder, and applied

directly to abscesses, nasal ulcers, and rheumatic joints. [17] One of the potential properties of the N. sativa seed is the ability of one or more of its constituents to reduce toxicity due to its anti-oxidant activities.[17]

The fruits of the date palm (phoenix dactylifera L-Areceae) are commonly consumed in many parts of the world and are a vital component of the diet in most of the Arabian countries. The dates, either fresh or dried, have high sugar content, low fats and protein contents, as well as iron, potassium and antioxidant flavenoids. Moreover, the utilization of date in medicine was reported. [18]

The aim of the present study was to study the pathological and biochemical effect of AFB_1 on the rat liver and kidney and to investigate the possible protective effect of the date fruits and *Nigella sativa* seeds on these organs.

Methods

Chemicals

AFB1 was obtained from Sigma-Aldrich (St. Louis, MO, USA). *Nigella sativa* oil with high degree of quality assurance was bought from the local market. Dates (Sokkare) were purchased from Qassim area. All other chemicals used were of the analytical grade.

Animal treatment

Forty healthy male albino rats (Rattus norvegicus) with average body weight 150–170 gm were used for this study. They were obtained from the Animal House of the Faculty of Medicine, King Saud University. All animals were conditioned at room temperature at natural photoperiod for 1 week before the start of the experiment. A commercial balanced diet and tap water ad libitum were provided. Animals were divided into four groups (10 animals each) and received the tested compounds daily for 2 weeks. The control group received notmal saline via intra peritoneal (I.P.) injection. The second group received AFB1 at a dose 50 µg/kg via I.P injection (19). The third group received AFB1 and Nigella sativa (NS) oil (6mg/Kg) via I.P. injection. The fourth group received AFB1 and aqueous date extract (15 gm/15 ml) by the oral route (gastric tube) (18). At the end of the experiment, the rats were scarified and livers and kidneys were excised immediately for histopathological studies.

The blood samples from all groups were collected from the orbital plexus of veins in heparinized tubes and were centrifuged at 5000 rpm for 10 min for plasma separation.

Histopathological Studies

The liver and kidney tissue samples were collected and fixed in 10% neutral buffered formalin and processed for histopathology using haematoxylin and eosin staining method according to Carleton et al. (20).

Biochemical parameters

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma were estimated by the method of King, (21). Protein content was determined by the method of Lowry et al. (22) using bovine albumin as a standard. The plasma levels of creatinine and urea were estimated according to Fabiny and Ertingshausen (23) and Chaney and Marbach (24) respectively.

Statistical analysis

The results are expressed as mean±standard error (SE). Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the Prism version 4 software package for Windows. The level of significance was *accepted* with P □0.05.

Results

Biochemical results:

As shown in Table 1, the plasma liver function indices, ALT& AST in AFB_1 group were significantly higher than control group. The levels of ALT and AST activities were reduced significantly in both AFB_1 and NS group and AFB_1 and date group in comparison with the AFB_1 group. The AFB_1 and NS group & AFB_1 and date group showed significant differences in the levels of transaminases activities.

On the other hand, the levels of creatinine and urea were significantly elevated in the AFB_1 group in comparison with controls. The levels of creatinine and urea were lower significantly in both AFB_1 and date group and AFB_1 and NS group in comparison with the AFB_1 group. The levels of total protein did not change significantly among different groups.

Pathological Results:

Gross picture: The liver of male albino rats, intoxicated with AFB_1 alone showed mild hepatomegaly in all animals. Severe haemorrhage and pale, depressed necrotic friable focal lesions were observed in seven cases and yellowish fatty areas with firm nodular structures were observed in three cases. Cut surface showed areas of congestion and haemorrhage. The kidney of the rats intoxicated with AFB_1 alone showed congestion, haemorrhage, swelling and yellowish-white and depressed areas. The liver as well as kidney of rats treated with AFB_1 and NS or AFB_1 and date showed nearly the same gross features. No morphological gross changes are detected either in liver or in kidney of the control group.

Microscopic picture: No remarkable histological changes were detected in the control cases either in liver or kidney. The liver of rats, treated with AFB_1 alone, showed severe pan lobular degenerative changes represented by cloudy swelling, vacuolar, hydropic and fatty degenerations in addition to diffuse necrosis of hepatocytes that were represented by nuclear pyknosis, karyorrhexis, cytoplasmic fine granularity and marked esinophilia. Congestion, haemorrhage, kupffer cell hyperplasia, interstitial edema with perivascular mononuclear cell infiltration were also detected (Fig.: 1-3). In three cases, isolated eosinophilic necrotic masses or patches were present with connective tissue proliferation and mononuclear cell infiltration “Nodular like grossly”. Portal tracts showed biliary hyperplasia, perivascular and interstitial edema, congestion and hemorrhage (Fig. 4-6). The kidneys of rats treated with AFB_1 alone, showed cloudy swelling, vacuolar, and hydropic degeneration in the tubular cells. Hyaline casts were seen inside the renal tubules that showed cystic dilatation in some sections, and necrotic changes in others. Renal tubules showed sloughed epithelial cells in their lumina and were surrounded by

polymorph nuclear leukocytes. Blood vessels showed dilataion, congestion and haemorrhage. Glomeruli showed cystic dilatation of glomerular Bowman's spaces, hypercelluarity of some glomeruli and sclerosis of others. Interstitial edema, fibrosis and heamorrhages were also observed (Fig.: 7-11). The liver of rats treated with AFB_1 plus NS oil showed less morphological changes, in comparison to those treated by AFB_1 alone. They showed few necrotic cells, biliary hyperplasia, congestion, perivascular as well as intercellular edema with kupffer cell hyperplasia. In two cases, the hepatocytes were nearly normal (Fig.12,13). Kidneys of male albino rats that treated with AFB_1 and NS, showed sub and capsular edema, congestion with perivascular edema and few mononuclear leukocytic cells infiltration. The renal tubules showed mild degenerative changes and few scattered necrotic debris (Fig.: 14). The liver of rats treated with AFB_1 and date were more or less normal. They showed mild congestion and kupffer cell hyperplasia (Fig.:15), whereas the kidneys of the same group were also more or less normal. They occasionally showed hydropic degeneration of the cells of proximal convoluted tubules, hyaline casts, congestion with few mononuclear cells infiltration (Fig. 16).

Discussion

The liver is the target organ for AFB_1. Ingestion of this mycotoxin, is know to be capable of inducing acute poisoning, aflatoxicosis, and is believed to be participated in the development of primary liver cancer. [25] AFB_1 was shown to be converted into its epoxide. This derivative produces DNA adduction causing DNA strand breaks and point mutations. [26] Moreover, under this pathological condition, the active process of cellular self-destruction, apoptosis, can occur. [27]

In the present study, the liver enzymes (ALT and AST) in AFB_1 -treated group were significantly higher than in the control group. This finding was confirmed by histopathological examination of liver tissues of AFB_1 -treated rats. The morphological changes in rat hepatocytes, in the current study, were previously described by many investigators. [28-31] The morphological changes included cloudy swelling, vacuolar, hydropic and fatty degeneration, diffuse necrosis as well as apoptosis of hepatocytes. Congestion, hemorrhage, kupffer cell hyperplasia, and biliary hyperplasia were also included.

On the other hand, AFB_1 -treated group showed higher levels of creatinine and urea than controls. This finding is keeping in line with findings of Rati et al [32] Histological examination of kidney tissues of the same group showed previously mentioned microscopic changes that were in agreement with the kidney dysfunction shown by biochemical tests.

Souza et al[33] reported that the oxidative stress is the principle manifestations of AFB_1–induced toxicity which could be mitigated by antioxidants. Previously, many researchers investigated the effect of different antioxidants such as melatonin on AFB_1. [27] Recently, Abdel-Wahhab and Aly [34] found that treatment with Nigella sativa oil of rats fed with aflatoxin-contaminated diet resulted in significant protection against aflatoxicosis. In the current study, the liver enzymes (ALT and AST) in AFB_1 and NS group were significantly higher than controls but these levels were reduced significantly in comparison with AFB_1 treated group. Histopathologically, the liver and kidney treated

with AFB_1 and NS showed less morphological changes in comparison to the changes seen in AFB_1 alone, which is an indication for partial protection.

The antioxidant effects of N. Sativa have been examined using different hepatic and kidney toxicity in vivo murine models induced by many toxic compounds such as carbon tetrachloride, tetra-butyl hydroperoxide, potassium bromate and schistosoma mansoni infection. [17] The N. sativa induced protection against hepatotoxicity via decreasing the elevated lipid peroxidation and levels of the liver enzymes and increasing the antioxidant enzymes levels. [35] Also Khan et al.[36] found N.sativa oil significantly decreases oxidative stress that coincide with marked recovery of renal glutathione content and antioxidant enzymes.

To the best of our knowledge, there are no previous studies exploring the effect of date on the aflatoxicosis. Date palms (Phoenix dactylifera L., Palmae) have been cultivated in the Middle East over at least 6000 years ago. [37] For the natives in this region, dates are considered a staple carbohydrate food. [38] Date fruits are also used in the production of local beverages and spirits. In local medicinal practices, dates are considered a "tonic" and "aphrodisiac", and in some communities they are thought to be useful against ulcer.[38] In fact, Muslims believe that "He who eats seven dates every morning will not be affected by poison or magic on the day he eats them". [39] Moreover, Vayalil [18] showed that date fruit has antioxidant and antimutagenic activity and this implicates the presence of compounds with potent free-radical-scavenging activity.

In the AFB_1 and date group, the plasma levels of liver function enzymes (ALT and AST), creatinine and urea were significantly lower than the AFB_1 group. Histopathologically, the livers and kidneys of rats treated with AFB_1 and Date, showed nearly normal and the mild changes were just vacuolation of hepatocytes, hyaline casts of renal tubules, congestion and few mononuclear cells infiltration.

The histopathological and biochemical actions of date may be due to its antioxidant effects. Vayalil [18] showed that the induced liver protection against aflatoxicosis occurred via decreased the level of liver enzyme activity as well as decreased the free radical propagation, also besides its lowering the pathological lesions resulted from AFB_1. The previous mentioned histological changes due to AFB_1 and NS as well as AFB_1 and Date were in agreement with those reported by many researchers.[18, 40-41]

Our study concludes that AFB_1 induced histopathological changes in the tissues of liver and kidney associated with dysfunction of these organs. Both NS and Date reduce the toxic effect of AFB_1 in liver and kidney which may be related to their cytoprotective and antioxidant properties, and their effect on some mediators of inflammation. The date treatment was most likely cytoprotective for liver than NS treatment against aflatoxicosis in rats.

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Table (1) Plasma bioindices of liver and kidney functions in different groups of male rats.

Variables	Controls	AFB1 group	AFB1 + Date group	AFB1 + NS group
Total protein (g/dL)	7.34±0.41	6.74±0.21 [a]P>0.05(NS)	7.19±0.26 [a]P>0.05(NS) [b]P>0.05(NS)	7.21±0.11 [a]P>0.05(NS) [b]P>0.05(NS) [c]P>0.05(NS)
ALT (U/L)	29.65±0.73	51.67±3.59 **[a]P<0.01**	37.38±0.83 **[a]P<0.001** **[b]P<0.01**	40.87±1.01 **[a]P<0.001** **[b]P<0.01** **[c]P<0.001**
AST (U/L)	25.88 ± 1.84	51.49 ± 2.08 **[a]P<0.001**	32.95 ± 2.12 **[a]P<0.05** **[b]P<0.001**	44.36 ± 1.04 **[a]P<0.001** **[b]P<0.01** **[c]P<0.001**
Creatinine (mg/dL)	0.814 ± 0.08	1.829 ± 0.19 **[a]P<0.001**	1.000±0.044 [a]P>0.05 (NS) **[b]P<0.001**	0.850±0.277 [a]P>0.05(NS) **[b]P<0.01** [c]P>0.05(NS)
Urea (mg/dL)	24.10±0.861	59.07±1.919 **[a]P<0.001**	50.63±3.383 **[a]P<0.001** **[b]P<0.05**	33.36±1.086 **[a]P<0.001** **[b]P<0.001** **[c]P<0.001**

Values are means±SE for 10 rats (N= 10 for each group). P values are shown as (a) for comparison AFB1-treated group, AFB1+Date group and AFB1+ NS group versus controls. (b) for comparison AFB1+ Date group and AFB1+ NS group versus AFB1-treated group. (c) for comparison AFB1+ Date group versus AFB1+ NS group. ALT, alanine transaminase; AST, aspartate transaminase; NS, Nigella sativa; AFB1, aflatoxin B1. Other details are given in materials and methods section.

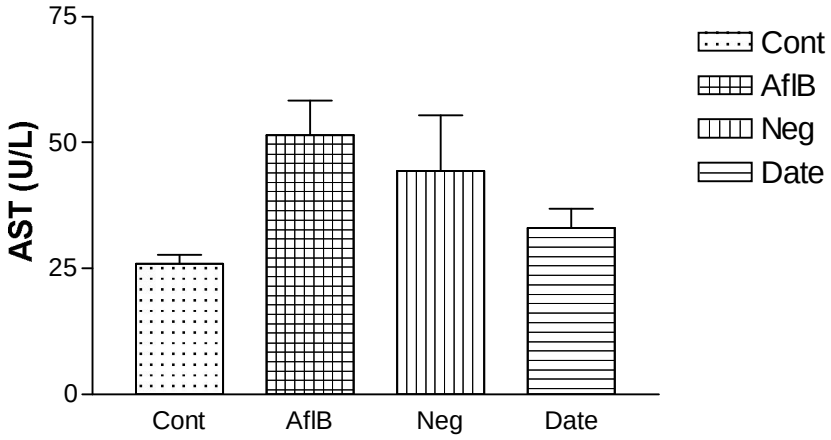

Fig. (1) Plasma levels of aspartate transaminase in controls and different treated rat groups.

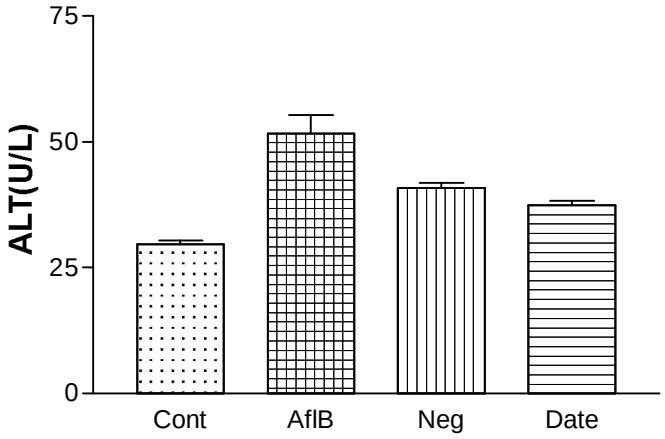

Fig. (2) Plasma levels of alanine transaminase in controls and different treated rat groups.

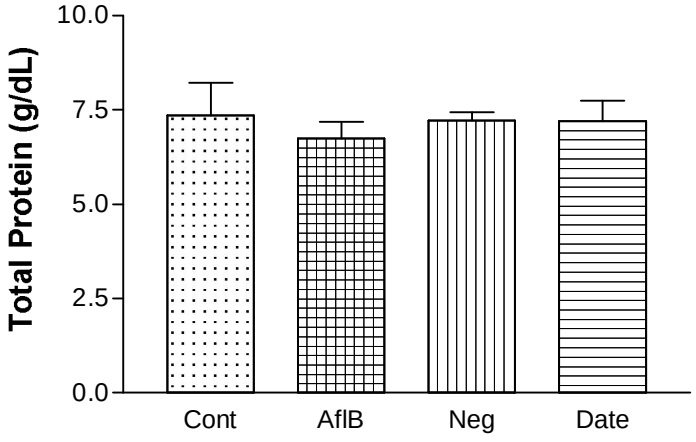

Fig. (3) Plasma levels of total protein in controls and different treated rat groups.

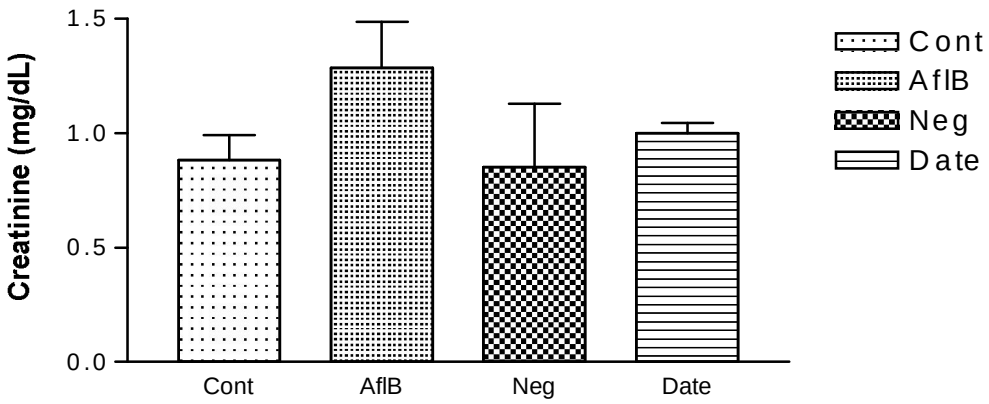

Fig. (4) Plasma levels of creatinine in controls and different treated rat groups.

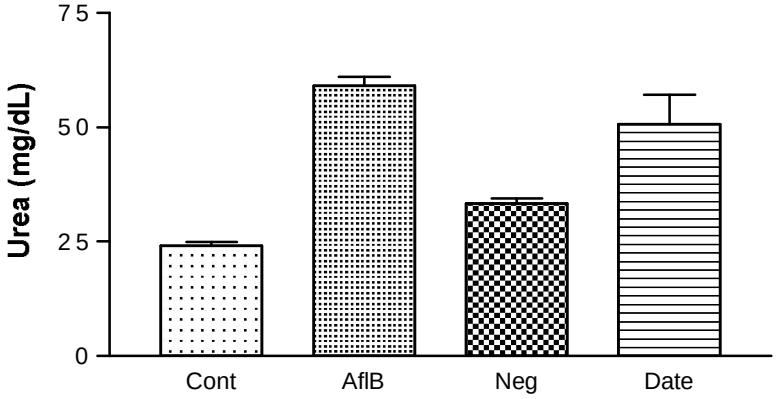

Fig. (5) Plasma levels of urea in controls and different treated rat groups.

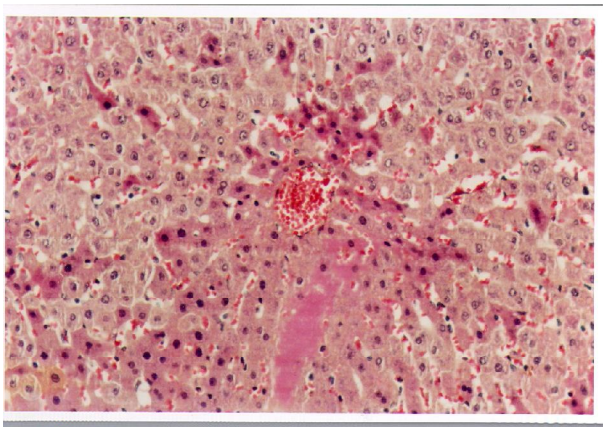

Fig 6: Liver of rats, intoxicated with AFB1, shows multifocal necrosis of hepatocytes, congestion and intercellular edema (H&E. X200).

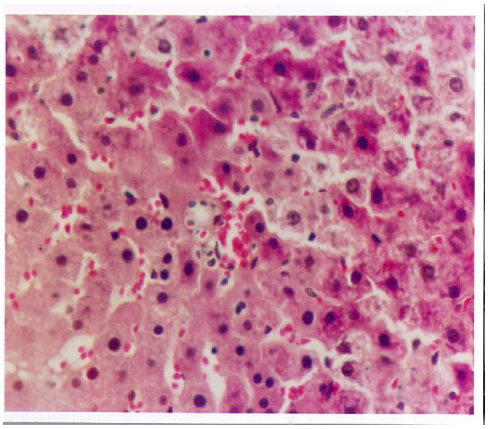

Fig. 7: Liver of rats, intoxicated with AFB1 , showing: diffuse hepatocellular necrosis with marked cytoplasmic eosinophilia.(H&E.X400).

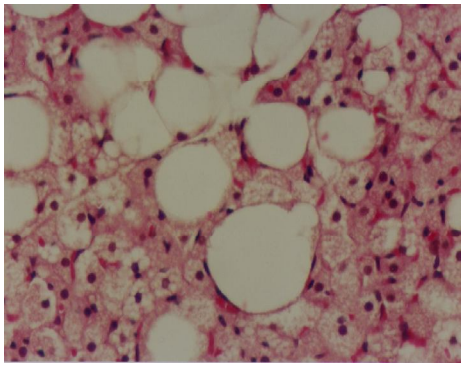

Fig.8: Liver of rats, intoxicated with AFB1 , showing: fatty and vacuolar degenerations of hepatocytes (H&E.X400)

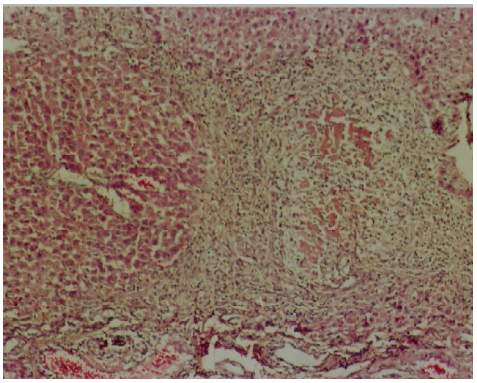

Fig 9: Liver of rats, intoxicated with AFB1 , showing normal liver cells to the left and hepatocellular necrosis and fibrous proliferation to the right (H&E.X 100)

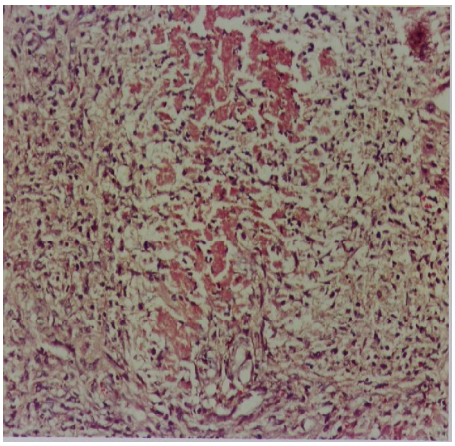

Fig. 10: Sequestration and fibrosis and mononuclear cells infiltrations (H&E.X200).

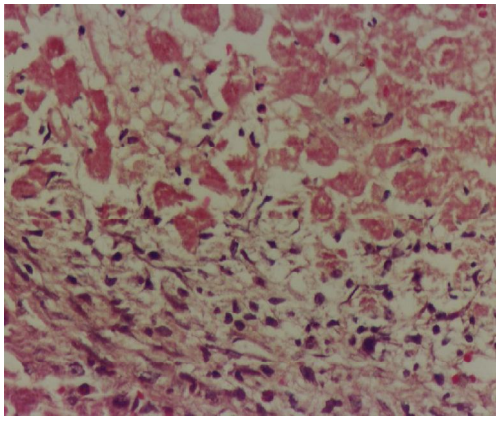

Fig. 11: Sequestration and fibrosis and mononuclear cells infiltrations (H&E.X400).

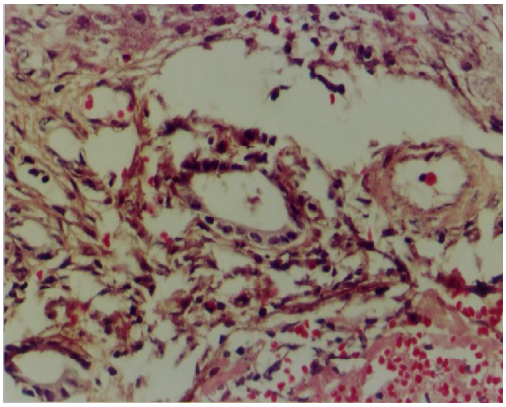

Fig.12: Liver of rat, intoxicated with AFB1, showing: biliary hyperplasia, congestion, hemorrhage, perivascular edema and mononuclear leukocytic cell infiltration (H&E.X400).

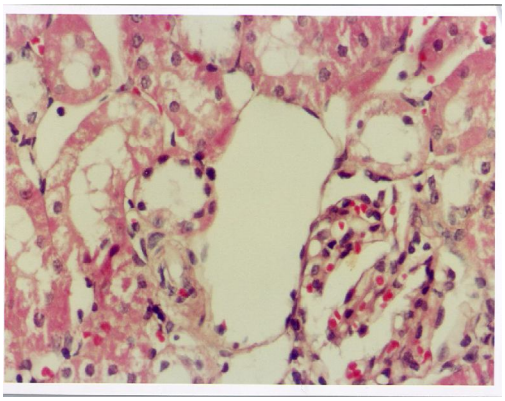

Fig. 13: Kidney of male albino rat, treated with AFB1 alone, showing cloudy swelling, vacuolar degeneration and necrosis of the renal tubular cells. Some tubules are cystically dilated (H&E.X400).

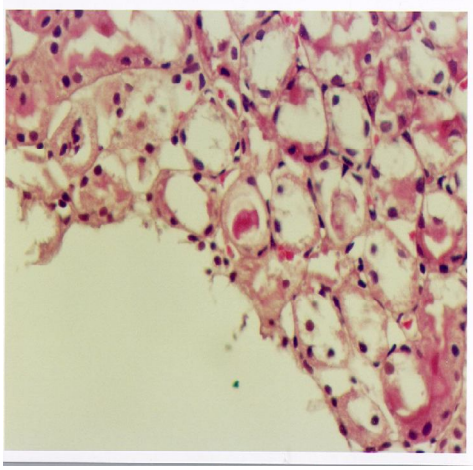

Fig. 14: Kidney of male albino rat, treated with AFB1 alone, showing hyaline casts and tubular necrosis (H&E.X400).

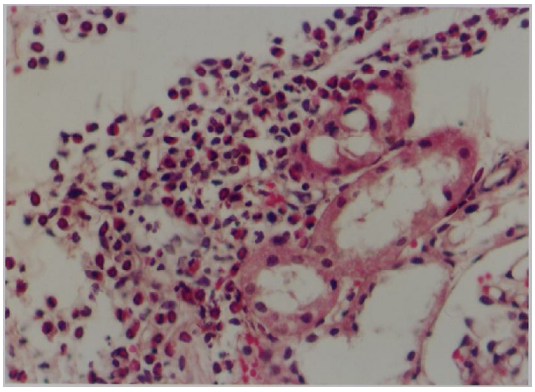

Fig. 15: Kidney of male albino rat, treated with AFB1 alone, showing: infiltration by eosinophilis and tubular necrosis (H&E.X400).

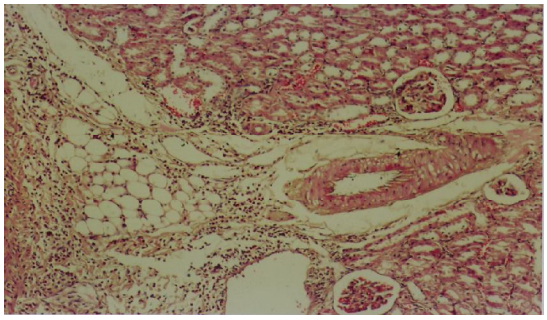

Fig. 16: Kidney of male albino rat, treated with AFB1 alone, showing fatty change, and inflammatory cell infiltration on top of tubular necrosis (H&E.X100).

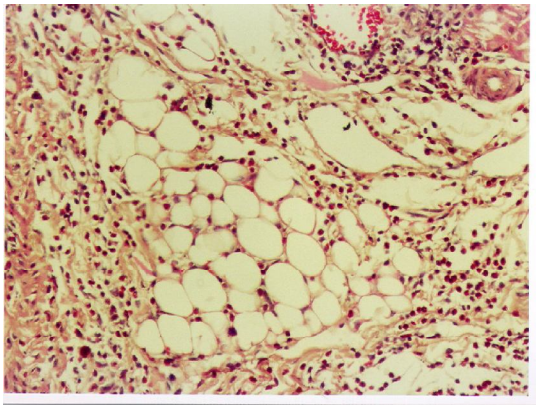

Fig. 17: Kidney of male albino rat, treated with AFB1 alone, showing fatty change, and inflammatory cell infiltration on top of tubular necrosis (a high power resolution).

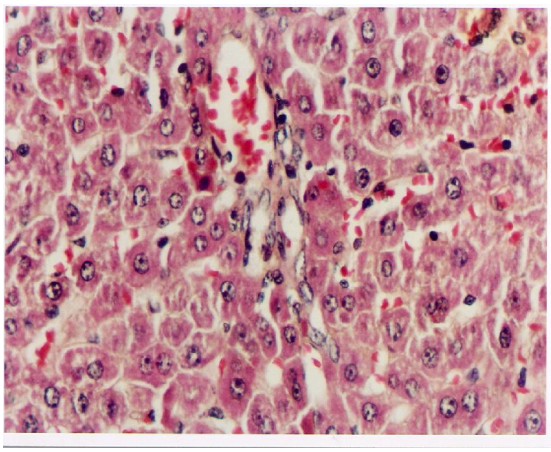

Fig. 18: Liver of male albino rat, treated with AFB1+N.sativa, showing:apoptotic hepatocytes and congestion (H&E.X400).

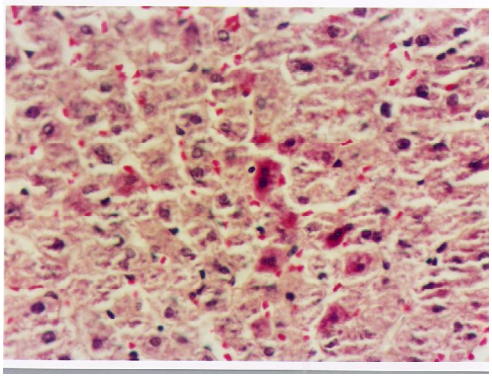

Fig. 19: Liver of male albino rat, treated with AFB1+N.sativa, showing focal liver cell necrosis (H&E.X400).

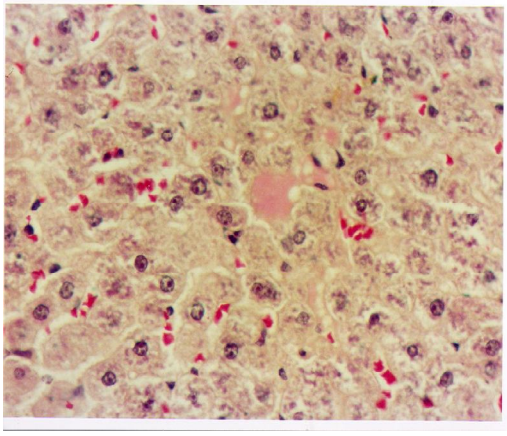

Fig. 20: Liver of male albino rats, treated with AFB1+N.sativa, showing mild degree of degeneration. Some hepatocytes contain multiple nucleoli (sign of regeneration and hyperplasia) (H&E.X400).

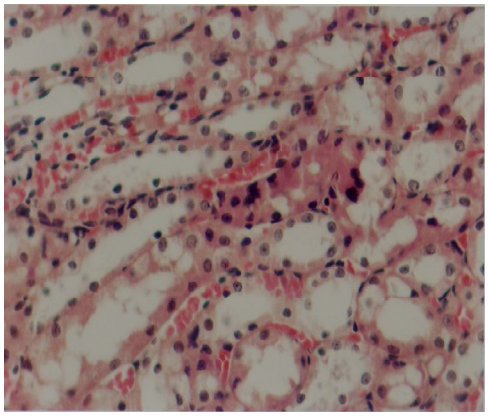

Fig. 21: Kidney of male albino rat, treated with AFB1+N.sativa, showing hydropic degeneration and congestion (H&E.X400).

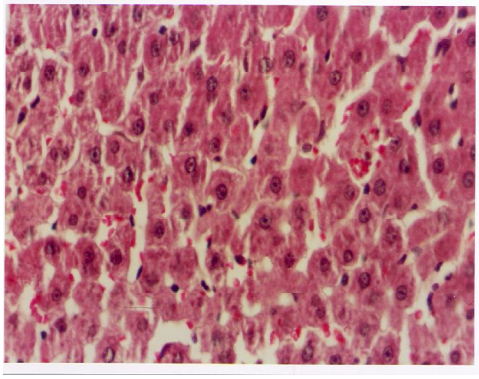

Fig. 22: Liver of male albino rat, treated with AFB1+Date, showing more or less normal liver except for mild congestion (H&E.X400).

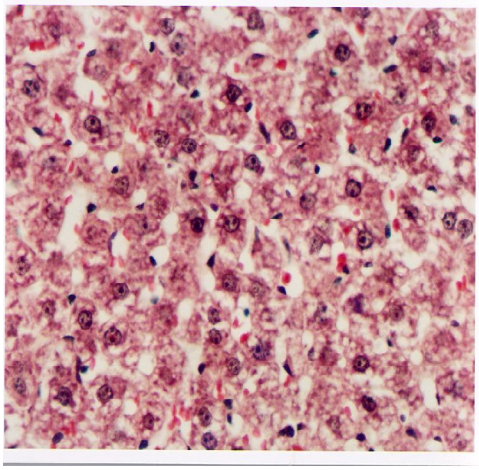

Fig. 23: Liver of male albino rat, treated with AFB1+Date, showing more or less normal hepatocytes “just mild degeneration”. (H&E.X400).

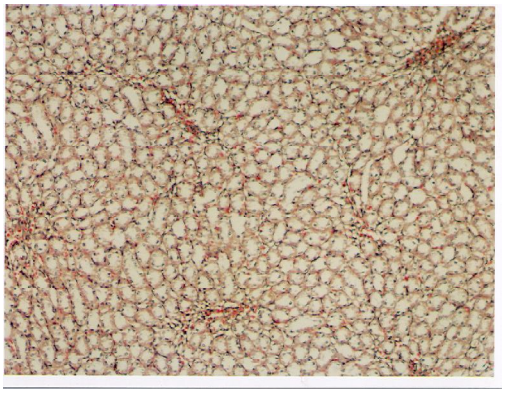

Fig. 24: Kidney of male albino rat, treated with AFB1+Date, showing nearly normal renal tubules (H&E.X200).