

ALLEVIATING THE HISTOLOGICAL ALTERATIONS OF SOME INTERNAL ORGANS OF RABBITS FED AFLATOXIN-B₁ CONTAMINATED DIET VIA *Nigella sativa* AND VITAMIN C

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ABSTRACT

This work was carried out to evaluate the alleviation ability of *Nigella sativa* (Ns) and or vitamin C for the toxic effect of aflatoxin B₁ in rabbits diet. Forty New-Zealand White male rabbits (average body weight 1000 ± 10 g) were used in five experimental groups (8 / group) for 6 weeks. The control group (T₁) fed control diet, 2nd group (T₂) fed the diet with 200 ppb aflatoxin B₁. The 3rd, 4th and 5th group (T₃, T₄ & T₅) fed diets with 200 ppb aflatoxin B₁ plus 1% Ns, 500 mg vitamin C/ kg diet and 1% Ns plus 500 mg vitamin C/kg diet, respectively. Results showed that Ns or vitamin C addition can alleviate the negative effect of aflatoxin B₁ on internal organs weight and histopathological lesions of liver and kidneys.

Keywords: Rabbits – Aflatoxin B₁ – *Nigella sativa* –vitamin C. – Histology.

INTRODUCTION

Acute aflatoxicosis causes hepatitis, hemorrhage, immune suppression, genetic damage (carcinogenicity, teratogenicity and mutagenicity) and death. Growth impairment and lowering of reproductive performance are the most sensitive clinical signs of chronic aflatoxicosis (Abdelhamid, 2000, 2003, 2005_{a,b} and 2009). Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Abdelhamid *et al.*, 1986, 1992a&b, 96, 98, 99a,b Abdelhamid, 1993 and Abdelhamid and Mahmoud, 1996). These techniques have not been used on a commercial scale due to high costs, the need for special facilities, losses of important nutrients and the questionable safety of chemical degradation products of aflatoxins.

The toxicity of aflatoxins may be strongly influenced by dietary chemicals that alter the normal responses of mammalian systems to these substances. A variable array of chemical factors, including nutritional components (e.g., dietary protein and fat, lipotropic agents, vitamins, and trace metals), food and feed additives (e.g. antibiotics and preservatives) as well as other chemical factors, may interact to modify the effects of aflatoxins in animals (Abdelhamid *et al.*, 1995a,b,c, Salem *et al.*, 2001 and Yousef *et al.*, 2003).

Vitamin is the most commonly used single supplement in many countries (Saubertlich, 1994). It is one of the most important reducing agents occurring in living tissue. While most animals synthesize their own vitamin C, humans and a few other animals, such as non-human primates and guinea pigs, do not have ascorbate accelerates hydroxylation reaction, in part by

donating electrons to metal ion cofactors of hydroxylase enzymes. Hydroxylation reactions are important in collagen synthesis, carnitine, conversion of dopamine to norepinephrine and in tyrosine metabolism. Ascorbate is also utilized to catalyze other enzymatic reactions, such as amidation necessary for maximum activity of the hormones oxytocin, vasopressin, cholecystokinin, and alpha-melanotropin (Levine, 1986). Ascorbic acid is a water-soluble, chain-breaking antioxidant which reacts directly with singlet oxygen, hydroxyl, and superoxide radicals. Proposed mechanisms of ascorbic acid activity include increasing the number of effectiveness of lymphocytes and enhancement of phagocytosis and of the immune system as well as prevention of cellular free radical damage. (Zaky *et al.*, 2000, Sahoo and Mukherjee, 2003 and Yousef *et al.*, 2003). The ability of vitamin C to stimulate the immune response and protection against bacterial infection has now been established in fish (Abdelhamid *et al.*, 1995a,b,c and Nayk *et al.*, 2007). Vitamin C alleviates the aflatoxin effect on rabbits (Salem *et al.*, 2001 and Yousef *et al.*, 2003) rats (Abd El-Mageed, 1987 and El -Daly *et al.*, 2005) guinea pigs (Netke *et al.*, 1997). *Nigella sativa* (NS) significantly reduced the negative effect of aflatoxin B₁ on pekkin duckling (Zaky *et al.*, 2000) and rats (Youssef and Ashry, 1999 and Abdelhamid *et al.*, 2002_{a&b} and 2005). The improvement by Ns may be due to its active compounds such as 1- nigellaone thymoquinone and thymohydroquinone which inhibit bacteria and improve body function and performance, 2- fat soluble unidentified factors and essential fatty and amino acids which display an essential role in growth performance, 3- several macro and micro elements which are responsible for regulating all vital functions in the body and improve the immunity, and 4- vitamins have essential role in growth performance (thiamin, riboflavin, pyridoxine and niacin) as mentioned by various authors (Mohan *et al.*, 1996; William, 1999; Seleem and Riad, 2005 and Seleem *et al.*, 2007) Also, may be due to its contents which regulate digestion and absorption and fight the internal parasites (Nasr *et al.*, 1996; Medenica *et al.*, 1997; Abdel-Azeem *et al.*, 1999 and Abd El-Hakim *et al.*, 2002).

The aim of the present study was two fold; first, studying the histotoxic effect of aflatoxin B₁ on liver and kidney of male rabbit, and secondly, examination of the ability of Ns and vit. C as antioxidant to prevent and ameliorate the marked histopathological alterations in liver and kidney induced by aflatoxin.

MATERIALS AND METHODS

This work was carried out in the Department of Animal Production, Fac. of Agric., Zagazig University, Egypt, in 2008-2009. Forty growing New Zealand white (NZW) male rabbits with average body weight of 1000 ± 10 g were randomly assigned to five groups (8 animal in each). The control group (T₁) fed a basal diet without aflatoxin B₁, 2nd group (T₂) fed basal diet with 200 ppb aflatoxin B₁. The groups 3rd, 4th and 5th fed basal diet with aflatoxin B₁ plus 1% Ns, 500 mg vitamin C / kg diet and 1% Ns + 500mg vitamin C/kg

diet, respectively. Vitamin C (20%) (United Co. For Chem. & Med. Prep., Egypt) was included at 2.5 g/ kg diet to obtain 500 mg vitamin C / kg diet.

Aspergillus flavus MD 341, was obtained from the Central Lab. of Residues in Agric. Products, Agric. Pesticides Res. Centre, Dokki, Egypt, for production of aflatoxin B₁ on liquid media (2% yeast extract and 20% sucrose). The aflatoxin concentration was determined using the method of A.O.A.C. (1990). The media was found to contain aflatoxin B₁ alone. The media sprayed on diet to obtain aflatoxin B₁ level required. Animals in each trial were housed in individual cages under the same managerial, hygienic and environmental conditions all over the experimental period. The formula and chemical composition of the basal diet are shown in Table (1). Daily fresh water was available all time and fed ad libitum basal diet and N.s bought from the focal market. At the end of the experimental feeding period, three animals from each group were fasted for 14- hours, slaughtered and different organs were weighed and proportionate to live body weight. specimens from liver and kidneys were collected and fixed in formalin solution (10%) for histological study. Paraffin sections of 6 microns thickness were prepared, stained with Haematoxylin and Eosin (Carleton *et al.*, 1980) and examined microscopically. Data of the trial were statistically analyzed using the General Linear model program of SAS (1996).

Table (1): Formulation and chemical composition (%) of the basal diet.

Ingredients	%
Yellow corn	17
Clover hay	35
Wheat bran	20
Barley	10
Soybean meal	13
Molasses	3
Sodium chloride	0.20
Methionine	0.20
Vitamins and minerals	0.30
Bone meal	1
Limestone	0.30
Chemical composition (DM basis)	
OM	89.70
CP	17.00
CF	16.50
EE	2.20
NFE	54.00
Ash	10.30

RESULTS AND DISCUSSION

1- Weight of internal organs:

Table (2) shows the effect of aflatoxin B₁ on internal organs weight of rabbits and its modification by *Nigella sativa* and vitamin C. The results showed that the aflatoxin diet with and without 1% NS, 500mg vitamin C/kg diet and 1% Ns + 500mg vitamin C/kg diets decreased the absolute weight of

liver, spleen, kidneys and lungs. The relative weight of these organs as % of body weight take the opposite trend. However, the weight (absolute and relative) of organs were not different significantly between treatments. these results agree with those of Nowar *et al.*, (1996) and Santurio *et al.*, (1999).

2- Histopathological examination:

The results of histopathological examination of the control group revealed that the liver (Fig. 1) and kidneys (Fig. 2) showed normal state. Liver of rabbits fed diet contaminated by aflatoxin B₁ characterized by focal necrosis in the hepatic paraenchyma (Fig. 3), associated with inflammatory cells infiltration and kupffer cells proliferation between the hepatocytes (Fig. 4). The portal area showed inflammatory cells infiltration mainly surrounding the bile duct with congestion in the portal vien (Fig. 5). Also, the effect of aflatoxin B₁ on kidneys caused an inflammatory cells infiltration which was detected in focal manner between the tubules and atrophied glomeruli (Fig. 6).

Table (2): The effect of aflatoxin B₁ on internal organs weight (g and %) of rabbits and its modification by vitamin C and Ns.

Items	Rations				
	Control	Aflatoxin B ₁ 200ppb	Aflatoxin B ₁ +1%Ns	Aflatoxin B ₁ + VC 500mg/kg diet	Aflatoxin B ₁ + 1% Ns+VC 500mg/kg diet
Liver					
g	73.25±9.65	66.62±5.61	58.07±3.57	52.95±10.27	55.97±6.23
%	3.55±0.41	4.69±0.24	3.87±0.17	3.52±0.54	3.89±0.2
Heart					
g	4.84±0.5	4.65±0.38	4.04±0.34	3.50±0.25	5.0±0.66
%	0.26 ±0.02	0.33±0.01	0.28±0.02	0.24±0.01	0.33±0.03
Spleen					
g	1.45±0.11	1.35±0.11	1.09±0.16	1.18±0.07	1.38±0.16
%	0.08±0.01	0.09±0.01	0.08±0.01	0.08±0.01	0.09±0.01
Kidneys					
g	13.76±1.18	12.89±1.29	10.07±0.54	10.52±0.49	10.66±0.92
%	0.74±0.04	0.90±0.07	0.69±0.03	0.74±0.07	0.70±0.05
Lungs					
g	12.50±0.98	12.24±0.79	10.92±1.91	10.21±0.99	10.09±0.66
%	0.68±0.04	0.88±0.07	0.74±0.12	0.70±0.04	0.67±0.07

Nigella sativa addition improved the histopathological lesions. Since, few focal inflammatory cells infiltration and kupffer cells proliferation in the hepatic paranechyma were observed (Fig 7). Also, there was slight congestion in the blood vessles associated with tubular degeneration in kidney (Fig. 8). The treatment with vitamin C reduced the hazard effect of aflatoxin B₁, since slight degeneration was noticed in the hepatocytes with hyperplasia in the bile ducts of liver (Fig. 9 & 10). In kidney, slight glomeruli swelling with hypertrophy (Fig 11), and medulla focal inflammatory cell infiltration were observed (Fig. 12).

The improvement of Ns plus vitamin C was better than Ns or vitamin C alone, since the portal area of liver showed slight inflammatory cells infiltration (Fig. 13) and diffuse kupffer cells proliferation between the hepatocytes (fig 14). Moreover no histopathological alteration were occurred in kidney (Fig.15).

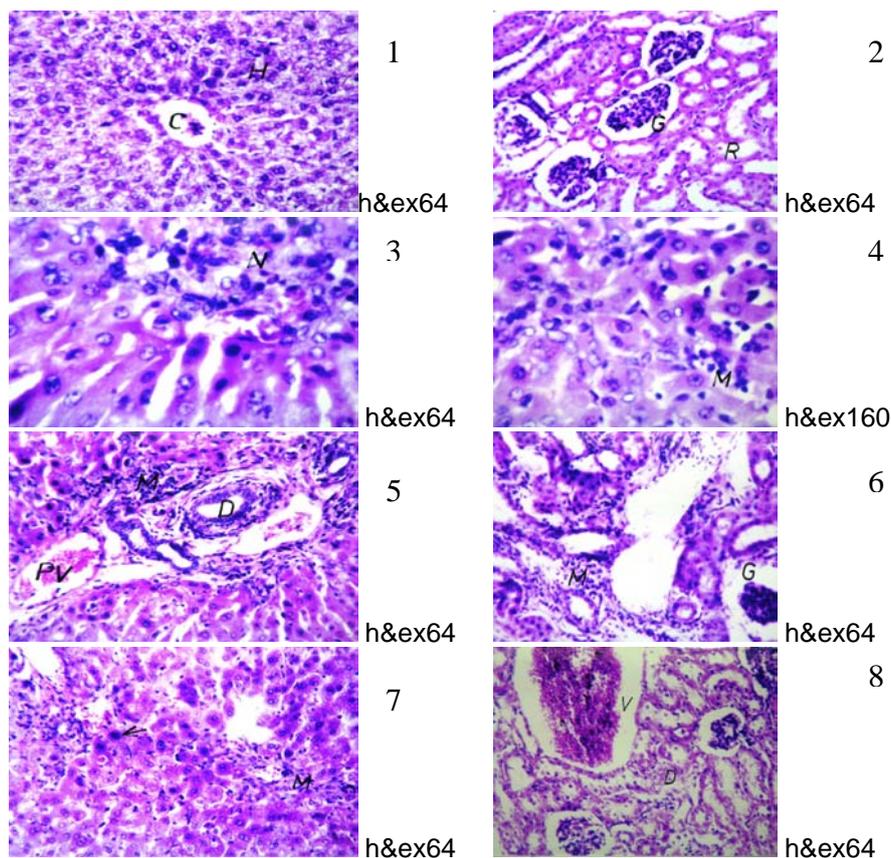


Fig.(1): Section in liver of control group showing no histopathological alteration and normal histological structure of the central vien and surrounding hepatocytes.

Fig.(2): Section in kidney of control group showing no histopathological alteration and normal histological structure of the glomeruli and tubules.

Fig. (3): Section in liver of aflatoxin group showing focal necrosis in the hepatic parenchyma.

Fig. (4): Section in liver of aflatoxin treated group showing inflammatory cells infiltration and kupffer cells proliferation between the hepatocytes

Fig. (5): Section in liver of aflatoxin group showing inflammatory cells infiltration in the portal area mainly surrounding the bile duct with congestion in the portal vien.

Fig. (6): Section in kidney of aflatoxin group showed inflammatory cells infiltration in focal manner between the tubules and atrophied glomeruli.

Fig. (7): Section in liver of aflatoxin plus *Nigella sativa* treated group showed few focal inflammatory cells infiltration and kupffer cells proliferation in the hepatic paraenchyma.

Fig. (8): Section in kidney of aflatoxin plus *Nigella sativa* treated group showed slight congestion in the blood vessels associated with tubular degeneration.

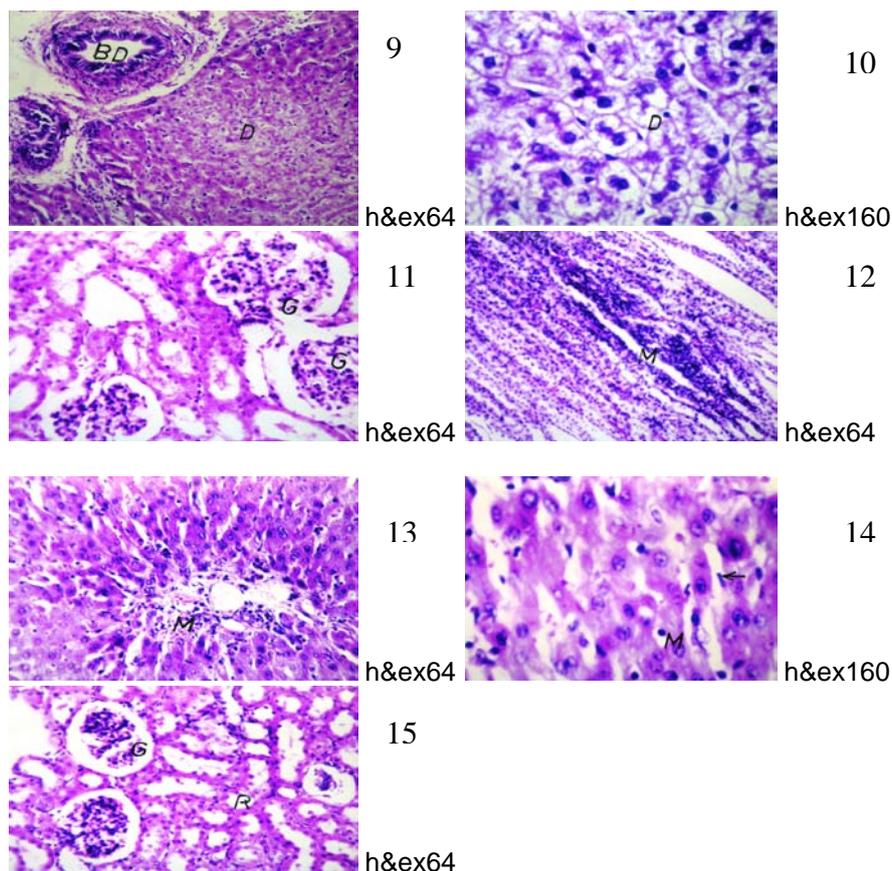


Fig. (9, 10): Section in liver of aflatoxin plus vitamin C treated group showed slight degeneration in the hepatocytes with hyperplasia in the bile ducts.

Fig. (11): Section in kidney of aflatoxin plus vitamin C treated group showed slight glomeruli swelling with hypertrophy.

Fig. (12): Section in kidney of aflatoxin plus vitamin C treated group showed focal inflammatory cells infiltration in medulla.

Fig. (13): Section in liver of aflatoxin with *Nigella sativa* plus vitamin C group showed slight inflammatory cells infiltration in the portal area.

Fig. (14): Section in liver of aflatoxin with *Nigella sativa* plus vitamin C treated group showed few inflammatory cells infiltration and diffuse kupffer cells proliferation between the hepatocytes.

Fig. (15): Section in kidney of aflatoxin with *Nigella sativa* plus vitamin C treated group showed no histopathological alteration.

Similar histological changes in liver and kidneys of rats fed 250 ppb aflatoxin B1 were reported by Abdelhamid *et al.* (2002_{a&b}) who found that hepatocytes arranged in thick plates with cellular pleomorphism and some nuclear hyperchromasia, and marked congestion of the glomerular capillaries

in kidneys. Samia Meshreky *et al.*, 2007 and found alteration in liver and kidney of rabbits treated with aflatoxin. Similar improving due to Ns were reported by Youssef and Ashry (1999) and Zaky *et al.* (2000).

Results of the present study demonstrated that adding 1% Ns or 500mg vitamin C/ kg diet alleviate the toxic effect of aflatoxin B₁. The improvement of Ns plus vitamin C was better than that of Ns or vitamin C alone in alleviation the hazard effect of aflatoxin B₁ in rabbits.

The present results indicated that treatment with 200 ppb aflatoxin B₁ induced histopathological changes in the liver and kidney. Liver showed inflammatory cells infiltration in the portal area mainly surrounding the bile duct with congestion in the portal vein, Kupffer cells proliferation between the hepatocytes, and necrosis in the hepatic parenchyma. Liver injury by aflatoxin was recorded by various investigators (Abdelhamid *et al.*, 2002_{a&b}, 2005 and El-Daly *et al.*, 2005). Kidney is also affected by aflatoxin B₁ and showed inflammatory cells infiltration in focal manner between tubules and atrophied glomeruli. These lesions were previously stated by (Abdel hamid *et al* 2002_{a&b}, 2005 and El- Daly *et al* 2005). The toxic effect induced by aflatoxin and manifested by marked histopathological lesions in liver and kidney was attributed to the mechanism of aflatoxin which inhibits protein synthesis and impaire nitrogen and energy utilization of the ingested diet through the adverse affects of aflatoxin on the liver, acenter of body metabolism also aflatoxin can bind with DNA and RNA and prevent the protein synthesis in the body. The haemorrhagic effect induced by aflatoxin was referred to its effect on clotting factors and resulted in incomplete synthesis of clotting factors (Nowar *et al.*, 1996). While necrotic effect of aflatoxin in hepatocytes may be attributed to the lock of key enzymes that support essential metabolism. Also, the toxic effect produced by aflatoxin on organs was explained by various investigators who demonstrated that, aflatoxin treatment resulted in enhancement of lipid peroxidation in rats, which is directly related to free radical mediated toxicity biomolecules such as nucliec acids, proteins and lipids. Antioxidants are believed to be important in health maintenance through the modulation of oxidative proceses in the body. Antioxidauts are know to reduce oxidative- radical induced reaction (Yousef *et al.*, 2003).

Histopathological examinations revealed that vitamin C reduced toxic effect of aflatoxin on liver and kidney. Whereas liver and kidney of rabbits treated with vitamin C and aflatoxins showed some degenerative changes but were less in severity than that in rabbits treated by aflatoxins alone. The beneficial influence of vitamin C is due to its being an important natural antioxidant which inhibits lipid peroxidation. Also, Yousef *et al.* (2003) stated that vitamin C is naturally occurring free radical scavenger and its presence anisted varoius other mechanisms in decreasing mumerous disruptive free radical process from taking place react directly with single oxygen, hydroxyl and superoxid radicals which could be caused by aflatoxin. Vitamin C also had antimutagenic effect and inhibits carcinogen- induced all transformation. Moreover, vitamin C suppressed the binding of aflatoxin hepatocyte DNA. The improvement by Ns may be due to its active compounds which are responsible for regulating all vital functions in the body and improve immunity. Also, may be due to its contents which regulate digestion and absorption and

fight the internal parasites. In conclusion Ns and vitamin C are capable of sustaining global antioxidants in liver and kidney cells leading to decreased oxidative stress and cellular damage initiate by aflatoxin B₁ through free radical production.

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تخفيف التأثيرات المرضية على أنسجة بعض الأعضاء الداخلية للأرانب المغذاه على عليقة ملوثة بالأفلاتوكسين B₁ باستخدام حبة البركة وفيتامين ج
أمل مصطفى أحمد - خالد مصطفى المليجي و منال عبد المطلع عطوة
المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية - وزارة الزراعة - مصر

إجرى هذا العمل ليقوم بقدرة حبة البركة وفيتامين ج على تخفيف سمية أفلاتوكسين B₁ في علائق الأرانب. فتم استخدام أربعين ذكر أرنب نيوزلاندى أبيض (متوسط وزن الجسم 1 كجم ± 10 جم). فى خمس مجموعات (8/مجموعة) لمدة 6 أسابيع. المجموعة الأولى غذيت على عليقة الكنترول ، المجموعة الثانية غذيت على عليقة تحتوى 200 جزء فى البليون أفلاتوكسين B₁، المجموعات 3، 4، 5، غذيت على عليقة تحتوى على 200 جزء فى البليون أفلاتوكسين B₁ بالإضافة الى 1% حبة بركة، 500 ملجرام، فيتامين ج ، 1% حبة بركة + 500 مجم فيتامين ج /كجم علف. وأنتهت النتائج إلى أن حبة البركة + فيتامين C خفف التأثير السلبى للأفلاتوكسين B₁ على وزن الأعضاء الداخلية والتأثيرات المرضية فى الكبد والكلية.

قام بتحكيم البحث

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