ALLEVIATING THE HISTOLOGICAL ALTERATIONS OF SOME INTERNAL ORGANS OF RABBITS FED AFLATOXIN-B$_1$ CONTAMINATED DIET VIA *Nigella sativa* AND VITAMIN C

Ahmed, Amal M.; Kh. M. El-Meleigy and Manal A. Atwa

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 B$_1$ 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 (T$_1$) fed control diet, 2$^{nd}$ group (T$_2$) fed the diet with 200 ppb aflatoxin B$_1$. The 3$^{rd}$, 4$^{th}$ and 5$^{th}$ group (T$_3$, T$_5$ & T$_5$) fed diets with 200 ppb aflatoxin B$_1$ 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 B$_1$ on internal organs weight and histopathological lesions of liver and kidneys.

Keywords: Rabbits – Aflatoxin B$_1$ – Nigella sativa –vitamin C. – Histology.

INTRODUCTION

Acute aflatoxicosis causes hepatitis, hemorrhage, immune suppression, genetic damage (carcinogenicity, teratogenicily and mutagenicity) and death. Growth impairment and lowering of reproductive performance are the most sensitine clinical signs of chronic aflatoxicosis (Abdelhamid, 2000, 2003, 2005a,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 (Sauberlich, 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
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donating electrons to metalion 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, cholecytkostatin, and alpha-melanotropin (Levine, 1986). Ascorbic acid is a water-soluble, chain-breaking antioxidant which reacts directly with singlet oxygen, hydroxyle, 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 pekkkin duckling (Zaky et al., 2000) and rats (Youssef and Ashry, 1999 and Abdelhamid et al., 2002a&b and 2005). The improvement by NS may be due to its active compounds such as 1- nigellaone thymoquinon and thymohydroquinon 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 Zeland 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 allover 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.**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>17</td>
</tr>
<tr>
<td>Clover hay</td>
<td>35</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20</td>
</tr>
<tr>
<td>Barley</td>
<td>10</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>13</td>
</tr>
<tr>
<td>Molasses</td>
<td>3</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamins and minerals</td>
<td>0.3</td>
</tr>
<tr>
<td>Bone meal</td>
<td>1</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Chemical composition (DM basis)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>89.70</td>
</tr>
<tr>
<td>CP</td>
<td>17.00</td>
</tr>
<tr>
<td>CF</td>
<td>16.50</td>
</tr>
<tr>
<td>EE</td>
<td>2.20</td>
</tr>
<tr>
<td>NFE</td>
<td>54.00</td>
</tr>
<tr>
<td>Ash</td>
<td>10.30</td>
</tr>
</tbody>
</table>

**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
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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 paraenchema (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 vian (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.

<table>
<thead>
<tr>
<th>Items</th>
<th>Rations</th>
<th>Control</th>
<th>Aflatoxin B1 200ppb</th>
<th>Aflatoxin B1 +1%Ns</th>
<th>Aflatoxin B1+ VC 500mg/kg diet</th>
<th>Aflatoxin B1 +1% Ns+VC 500mg/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver g</td>
<td></td>
<td>73.25±9.65</td>
<td>66.62±5.61</td>
<td>58.07±3.57</td>
<td>52.95±10.27</td>
<td>55.97±6.23</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>3.55±0.41</td>
<td>4.69±0.24</td>
<td>3.87±0.17</td>
<td>3.52±0.54</td>
<td>3.89±0.2</td>
</tr>
<tr>
<td>Heart g</td>
<td></td>
<td>4.84±0.5</td>
<td>4.65±0.38</td>
<td>4.04±0.34</td>
<td>3.50±0.25</td>
<td>5.0±0.66</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>0.26±0.02</td>
<td>0.33±0.01</td>
<td>0.28±0.02</td>
<td>0.24±0.01</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>Spleen g</td>
<td></td>
<td>1.45±0.11</td>
<td>1.35±0.11</td>
<td>1.09±0.16</td>
<td>1.18±0.07</td>
<td>1.38±0.16</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>0.08±0.01</td>
<td>0.09±0.01</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Kidneys g</td>
<td></td>
<td>13.76±1.18</td>
<td>12.89±1.29</td>
<td>10.07±0.54</td>
<td>10.52±0.49</td>
<td>10.66±0.92</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>0.74±0.04</td>
<td>0.90±0.07</td>
<td>0.69±0.03</td>
<td>0.74±0.07</td>
<td>0.70±0.05</td>
</tr>
<tr>
<td>Lungs g</td>
<td></td>
<td>12.50±0.98</td>
<td>12.24±0.79</td>
<td>10.92±1.91</td>
<td>10.21±0.99</td>
<td>10.09±0.66</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>0.68±0.04</td>
<td>0.88±0.07</td>
<td>0.74±0.12</td>
<td>0.70±0.04</td>
<td>0.67±0.07</td>
</tr>
</tbody>
</table>

Nigella sativa addition improved the histopathological lesions. Since, few focal inflammatory cells infiltration and kupffer cells proliferation in the hepatic paranechema 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.
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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.* (2002a,b) who found that hepatocytes arranged in thick plates with cellular pleomrphism 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., 2002a&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. 2002a&b, 2005 and El-Daly et al. 2005). The toxic effect induced by aflatoxin in rabbits was 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, a center 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 nucleic acids, proteins and lipids. Antioxidants are believed to be important in health maintenance through the modulation of oxidative processes in the body. Antioxidants 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 various other mechanisms in decreasing numerous disruptive free radical process from taking place react directly with single oxygen, hydroxyl and superoxidad 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.

REFERENCES


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