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Effect of Aflatoxin B1 (AFB1) Residues on the Pathology of Camel Liver

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ABSTRACT

This study was carried out to evaluate the potential effect of high Aflatoxin B1 (AFB1) residues on the pathology of livers of camels slaughtered at different abattoirs in AL-Ahsa region, Kingdom of Saudi Arabia. Aflatoxin residues were determined in a total number of 160 camel liver samples. Thirty seven (23.1%) liver samples showed residues of AFB1 higher than the standard permissible limit (spl. 0.05 ppb). Seventeen of these samples contained very high residues between 0.1-1.0 ppb, while twenty samples contained residues between 0.05-0.1 ppb. However, 123 (76.9%) liver samples showed AFB1 residues less than the standard permissible limits (spl). Small portions of the liver tissue from all animals were removed, weighed and preserved in 10% formalin solution and then embedded in paraffin wax. Pathological changes were observed in the liver tissues collected from all camels. Liver samples containing high AFB1 residues showed remarkable gross changes including fatty degeneration with variable areas of petechial hemorrhages, Congestion, fibrosis and large whitish focus of necrosis. However, mild gross changes were obtained in the liver samples containing medium AFB1 residues, between (0.05->0.1 ppb.) and no gross changes were obtained in the liver samples containing AFB1 residues below the standard permissible limits (spl). In addition the histopathological changes in the liver samples with high residues of AFB1 showed vacuolar degenerations, cholangitis, cirrhosis, bile duct carcinoma and hepatocellular carcinoma while hepatocytic fatty vacuolation, hydropic degeneration, congestion and mild degree of peribiliary cirrhosis were observed in the liver samples containing medium, AFB1 residues and no remarkable changes were seen in the liver samples containing AFB1 residues below the standard permissible limits (spl). These results revealed that aflatoxin residues may cause massive histopathological changes to the liver tissue of camels and caution should be exhibited in its use for human and animal consumption.

Key words: Aflatoxin, residue, camel liver

INTRODUCTION

Aflatoxins are considered the most feed contaminations in Saudi Arabia, due to the favorable environmental conditions (Bokhari, 2010). International Agency for Research on Cancer (IARC) has classified Aflatoxins as carcinogenic to humans. It has been shown that 40% of liver tissue cancer in Africa is caused by aflatoxins (Williams *et al.*, 2004). Samples of camels blood sera collected from camels died in Abu Dhabi were found to contain aflatoxins ranging from 5-50 ng mL⁻¹ total

aflatoxin and 28 out of the 37 samples were found to contain aflatoxin at the range of 2-12 $\mu\text{g mL}^{-1}$ total aflatoxins (Osman and Abdel-Gadir, 1991). Abbas and Ali (2001) reported that Camels with aflatoxicosis had a mean concentration of retinol in the plasma of $243.4 \pm 32.3 \text{ ng L}^{-1}$. The concentrations of aflatoxins in the liver and ruminal contents of these animals were 18.2 and $243.4 \mu\text{g kg}^{-1}$, respectively. Aflatoxin (AFB1) residues were observed in the livers of different species. They recorded residue levels of 0.12 ppb in camels, 0.051 ppb in beef calves, 0.015 ppb in buffalo calves and 0.015 ppb in sheep (El-Shewy *et al.*, 1997; Dangelo *et al.*, 2007). Hence, this present study was carried out to evaluate the residue levels of aflatoxin in camel liver tissues and to study the pathological alterations in camel liver samples as a result of high residues of aflatoxin.

MATERIALS AND METHODS

Histopathological techniques: Liver samples were collected randomly from 160 camels under study. These samples were fixed in 10% neutral formalin, mounted in paraffin, sectioned and stained with Haematoxyline and Eosin (HE) according to the method of Bancroft and Gamble (2008).

Determination of aflatoxin residue in the liver: Analysis of aflatoxin residue in the liver samples were carried out using double plate automatic biomedical system (Switzerland 2012), following ELISA protocol described in BIOO scientific (2008). The obtained results were compared with the permissible limit according to Saudi Arabian Standards Organization (SASO., 1998).

RESULTS

Table 1 shows the ranges of AFB1 (ppb) in a total number of 160 samples of camel livers collected from different abattoirs in Al Ahsa region. One hundred twenty three samples (76.9%) contained residues less than the maximum acceptable levels or standard permissible limits ($0.05 \mu\text{g kg}^{-1}$). Twenty cases (12.5%) contained levels between ($0.05 \geq 0.1$) and 37 cases showed higher levels ranging from ($0.1 \geq 1.0$).

Gross changes: About 25 (15.6%) camels with high residues revealed various degrees of gross changes in the liver (Fig. 1a-b, Table 2).

Table 1: Residue levels of aflatoxin in camel liver tissues (ppb)

Levels of AFB1	Range of AFB1 (ppb)	Liver samples	
		No.	%
Low	$0.00 \geq 0.05$	123	76.9
Medium	$0.05 \geq 0.1$	20	12.5
High	$0.10 \geq 1.0$	17	10.6

Table 2: Summary of necropsy findings in camel livers containing AFB1 residues

Findings	Group 1 high AFB1 level	Group 2 medium AFB1 level	Group 3 low AFB1 level
Congestion	++++	++	+
Hemorrhage	++++	++	-
Fatty change	++++	+	-
Necrosis	++++	+	-
Cirrhosis	++++	+	-

+, +++++: Increase in severity, -: Absence of lesions



Fig. 1(a-b): (a) Enlarged fatty liver with variable areas of petechial hemorrhages and (b) Congestion, grossly pseudo lobulated liver with the fibrotic bands and large whitish focus of necrosis

Histopathological findings: Figure 2a-d shows the photomicrograph of the liver of camels containing high levels of AFB1 (0.05-1.0 ppb). Figure 2e-f shows photomicrograph of the livers of camels containing, medium level of AFB1 (0.05-0.1 ppb) and Fig. 2g-h shows the photomicrograph of the liver of camels containing low levels of AFB1 ($0.00 \geq 0.05$ ppb).

DISCUSSION

The present study was conducted to investigate the pathological, changes related to AFB1 residues in the camel livers. A total of 160 camel livers were collected from different abattoirs and veterinary clinics in AL-Ahsa region kingdom of Saudi Arabia. AFB1 residues in these livers were measured and then all liver samples were subjected to pathological studies.

The detected residue levels of AFB1 in liver samples were compared to the permissible limits (0.05 ppb), stated by the Saudi Arabian Standards Organization (SASO., 1998).

One hundred and twenty three (76.9%) camels showed residue levels under the permissible limit. While 37 (23.1%) camels showed residue levels higher than the permissible limit. These results are nearly in agreement with the results obtained by El-Shewy *et al.* (1997) in camel liver and also comparable to the values reported in other animal species (Stubblefield and Shotwell, 1981; Hammad, 1995; Dangelo *et al.*, 2007). On the other hand, the present data of residue levels are not consistent with some other reports in other countries (Abbas and Ali, 2001; Osman *et al.*, 2004). This difference may be attributed to the outbreaks of toxicity with AFB1 in these studies.

Gross examination for the livers in the present study showed changes, especially in those samples with high AFB1 residues. These lesions were mainly in the form of swelling and enlargement of the liver with presence of large areas of pale colorations of fatty liver, congestion,

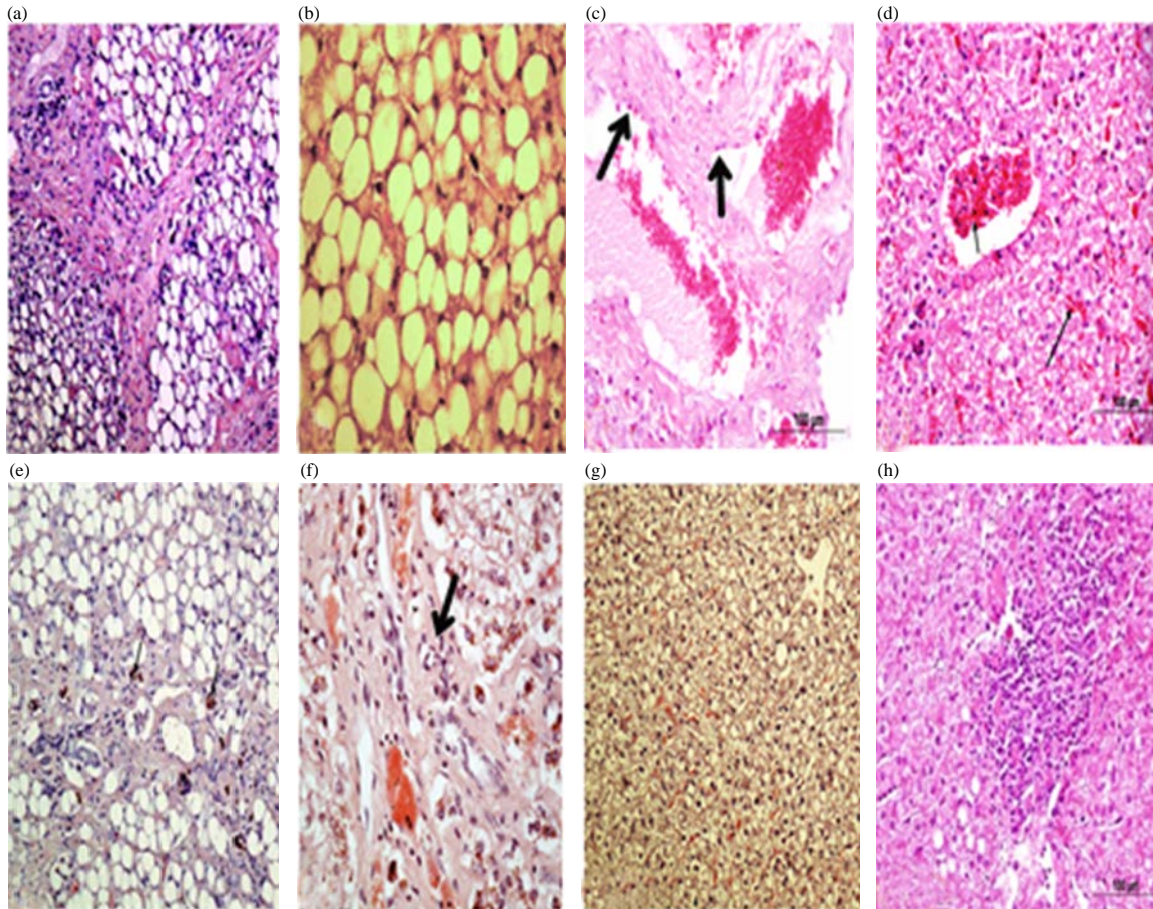


Fig. 2(a-h): (a-b) Severe degree of hepatocytic fatty change with pseudo lobulations and cirrhosis (H and E = X400), (c-d) Variable sized fatty vacuolations of the hepatic cells, areas of large wide blood spaces of cavernous haemangioma with an excess of interstitial fibrous tissue proliferations and fibrosis and bile duct carcinoma (arrows) (H and E = X600), (e-f) Wide area of hepatocytic fatty vacuolation and hydropic degeneration portal, congestion and some golden bile pigments and mild degree of peribiliary cirrhosis (arrow) (H and E = X400) and (g-h) Small to large hepatocytic fatty vacuolation with mild congestion of some sinusoids and central vein with mononuclear cell infiltration (H and E = X100)

petechial hemorrhages, necrosis and cirrhosis. Most of these gross findings were nearly similar to those lesions described in camels aflatoxicosis cases (Osman *et al.*, 2004) and also similar to those lesions reported in other species (Smith *et al.*, 1976; Dangelo *et al.*, 2007; Kaleibar and Helan, 2013).

The microscopic changes in the liver specimens in the present study have indicated variable changes mostly correlated to the levels of AFB1 residues. The gradual severity of these microscopic alterations were positively proportional to the increase in the residue levels of AFB1. The microscopic alterations in liver samples with lower levels were mostly in the form of congestion in

the sinusoidal and central vein, hepatocytic steatosis and fatty vacuolations, vacuolar to hydrobic degenerations and haemosidrine pigments. Nearly similar histopathology alterations were previously reported in some studies for aflatoxicosis in other species as these of Colakoglu and Donmez (2012) in Rams, Smith *et al.* (1976) in farm animals, Armbrecht *et al.* (1970) in sheep, Miller *et al.* (1984) in goats, Hinton *et al.* (2003) in rat and Ortatatli *et al.* (2005) in broilers.

In liver samples with higher AFB1 residues the histopathological changes were more severe. They included diffuse changes of fibrosis, cirrhosis, cavernous haemangioma, bile duct carcinoma and hepatic carcinoma were seen. These lesions were similarly reported in other outbreak in camel aflatoxicosis (Osman *et al.*, 2004) and in other species (Vaid *et al.*, 1981; Van Halderen *et al.*, 1989; Hussain *et al.*, 2008; Pierezan *et al.*, 2010; Mursal and Saad, 2010).

The changes of haemangioma are mainly due to severe hyperaemia in the hepatic sinusoids and arteriole that were severely dilated and become susceptible to the neoplastic changes (Unal *et al.*, 2012). The bile duct hyperplasia and carcinoma may be due to the direct effect of aflatoxin on the cell lining of the bile ducts or due to the production of prostaglandin during peroxidation of lipids (Quist *et al.*, 2002; Saif *et al.*, 2003). The hepatocytic carcinoma can be caused by the effect of aflatoxin metabolites that arising from the effects of some of the enzymes which react with hepatocyte DNA that thought to be lead to mutations in the nuclei (Shen *et al.*, 1995).

CONCLUSION

The AFB1 residue levels higher than the permissible limits were detected in the livers of slaughtered camels in Al-AHsa region in Saudi Arabia.

The high residues of AFB1 in the camel liver were associated with some severe histopathological alterations in the liver manifested by degenerative changes, cirrhosis, bile duct carcinoma as well as cavernous haemangioma.

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