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CLINICO-DIAGNOSTIC STUDIES ON AFLATOXICOSIS IN CAMELS AS A FIELD PROBLEM

(With 3 Tables and 8 Figures)

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دراسات إكلينيكية لتشخيص التسمم بالأفلاتوكسين

فى الجمال كمشكلة حقلية

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فى إبريل ١٩٩٦ تلاحظ على بعض الجمال بمزرعة العوينات بالوادى الجديد فقدان فى الشهية وخمول و ورم تحت الفك مع تضخم فى الغدة الليمفاوية تحت الفكين بالإضافة لتقرحات فى اللثة ونفاخ وفى بعض الحالات المتقدمة ظهرت أعراض عصبية فى صورة شلل فى القوائم الخلفية والأمامية وغالباً ما تنتهى هذه الأعراض بالوفاة بعد ٥ - ١٠ أيام من ظهور الأعراض وقد كانت معدلات الوفاة ٣٣ ٪ ، وبفحص إحدى الحيوانات النافقة وجد نقط نزفية فى طبقة الدهون الموجودة تحت الجلد مع إحتقان فى جميع الأحشاء الداخلية كما وجد تضخم وإحتقان واضح فى الكبد وهشاشة فى نسيجه مع ظهور خطوط صفراء بالإضافة إلى إحتقان شديد بالأوعية الدموية فى المخ. وبينما كانت نتائج فحص العينات بالمسببات الفيروسية والبكتيرية وفحوص طفيليات الدم سلبية أمكن عزل فطر الأسبريجلس وسموم الأفلاتوكسين التى يفرزها بنسبة عالية (١٠٠ جزء فى البليون) من عينات البرسيم والدريس والتبن التى تتغذى عليها هذه الجمال. وقد تم فحص عدد ٢٢ عينة سيرم من الجمال المريضة حيث أوضحت النتائج إرتفاع معنوى فى نشاط كل من الفوسفاتيز القلوى والألانيين ترانسفيريز وكذا الأسبرتات ترانسفيريز مع إنخفاض مستوى كل من البروتين والبولينا وحامض البوليك والكرياتينين مما يوضح التأثير الكبير لوظائف الكبد تحت تأثير الأفلاتوكسين ، وحيث أن الكبد هو العضو الأساسى الذى يحدث فيه هضم وتخزين وإخراج مثل هذه السموم فقد أمكن إستبيان وقياس الأفلاتوكسين بجميع مشتقاتها. وقد أوضح الفحص الهستوباثولوجى وجود تغيرات تحللية وتنخر فى خلايا الكبد والأمعاء وألياف عضلة القلب وبعض الخلايا العصبية كما كان الإحتقان والنزيف من أهم السمات العامة لجميع الأعضاء التى تم فحصها وقد تم عرض النتائج بالتفصيل ومناقشتها .

SUMMARY

In late spring of 1996 a field problem of aflatoxicosis in Camels was detected in El Ewaynate Farm at El-Wady El-Gadid distrect. Where the clinical signs were pyrexia, loss of appetite, dullness, submandibular odema, enlargement of submaxillary lymph node, ulceration of the upper gum and tympany. In advanced cases nervous manifestations, tremors and paralysis of fore and hind quarter were observed. Death occured after 5-10 days from the onset of clinical signs. The mortality rate was 33%. The postmortem examination showed echymotic and petechial haemorrhage in subcutaneaus fat as well as congestion of the all internal organs. The liver was enlarged, congested with appearance of yellowish streakes and was friable in texture. The meningeal blood vessels were congested. Mycotoxicosis has been incriminated as a probable cause while bacterial , parasitic and viral examination of all collected samples revealed -ve results .The offered food samples (green & dried alfalfa) were examined for the presence of aflatoxins. The results indicate the presence of high concentration of aflatoxins (100 p.p.b). Toxogenic aspergellus flavus strain was isolated from the alfalfa. Examination of 22 serum samples revealed a pronounced elevation in the activities of alkaline phosphatase, Alanine and Aspertate transanimises with decrease in total proteins, urea, uric acid and createnine levels which indicates the great affection of the liver functions due to aflatoxicosis. Aflatoxins also were detected in the liver tissues (G1 83.07, B1 66.7, G 2 11.3 and B2 33.6 p.p.b.). Histopathological examination showed degenerative changes and necrosis in hepatocytes, omasum, abomasum, intestinal villi, cardiac muscle fibers and some neurons of cerebrum. Congestion and heamorrages were the predominant feature in all examined organs. The obtained results were illustrated and discussed.

Key words: Aflatoxins - Camels - Diagnosis

INTRODUCTION

Recently great attention was paid towards the existence of aflatoxins in food, because of their acute toxic and hepatocarcinogenic

effects in human and animals (Balata and Bahout, 1996). Aflatoxins are natural metabolites produced by toxogenic strains of *Aspergillus Flavus* and *Aspergillus Parasiticus* when contaminate feed stuffs of livestock and poultry under certain temperature and relative humidity. (Kisza and Demagala, 1994). Growth of these two toxigenic strains often results in injurious levels of aflatoxins mainly aflatoxin B_1, the most biologically active member of the aflatoxin family.

Hay and forage may be sources of mycotoxins including aflatoxins (Pier, 1992). Mycotic contamination of the green foods in the pasture may also occure as recorded by Alessi et al (1994).

Effects of aflatoxin consumption are similar in all animals(Edds, 1973). However the animals susceptibility to aflatoxin varies by species, age and individual variation (Pier, 1987). Low concentrations of aflatoxins in the feed have been reported to cause weakness, decreased resistance to diseases and have induced carcinogenesis in many species, while acute aflatoxicosis has been characterized by hemorrhage in many tissues, hepatotoxicosis with icterus and death (Edds, 1973).This work was done to study the biochemical and histopathological changes in camels affected with aflatoxicosis as a field problem after feeding green and dried alfalfa heavely contaminated with aflatoxin.

MATERIAL and METHODS

Case history

In late spring (April, 1996) 21* out of 63 one humped camels (camelus dromedaries) of different ages and sexes died after sudden onset of nervous manifestations mainly in the form of incoordination movement and paralytic like signs in the hind limbs, loss of reflexes and animals were off food. Some animals had tympany, submandibular odema, enlargement of submaxillary lymph node, ulceration of the upper gum..

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* In El Ewaynat Farm at El Wadi El Gadid, Egypt.

Samples:

(I) Random samples of alfalfa, hay and wheat straw were collected in clean polyethylene bags to be examined for the presence of aflatoxin contamination using thin layer chromatography (Bauer et. al., 1981), and Isolation and identification of fungi using the method described by Refai (1979).

The purified fungal isolates were examined macroscopically and microscopically. Aspergilli were identified according to Raper and Fennel (1965). For identification of other fungi, Medical Mycology Manual was used as a guide (Beneke and Rogers, 1970). Determination of the toxicity of isolated *Aspergillus Flavus* strains was carried out according to Schrocder and Boller (1973), where the media for quantitative determination of aflatoxin production consisted of 39 of ground straw, hay and alfalfa from the collected samples in 50 ml of water per 250 ml Erlenmeyer Flask. Asemiliquid slurry resulted after sterilization in an auto clave for 20 min. at 15 lb/in^2 . Each Flask was inoculated with a loop of spores scraped from a slant of the respective culture, shaked thoroughly to disperse the spores and thus loster the development of uniform colony growth, and then incubated for 7 days at 25°C. Also yeast extract sucrose broth (2% yeast extract, 15% sucrose) was inoculated by the spores of the isolated strains. The aflatoxins were extracted by chloroform and estimated by TIC according to Bauer et al. (1981).

(II) 22 Blood samples were taken through the jugular vein in clean, dry test tubes and clear sera were seperated to be used for :-

Estimation of Alanine and Aspartate amino transferases (ALT & AST) [Reitman and frankel, 1957] , Alkalin phosphatase (Kliching and Freiburg, 1951] , total proteins (Weichselbaum, 1946) , uric acid (Artiss and Entwistle , 1981) , Urea (Patton and Crauch , 1977) and Creatinine (Husdan and Rapoport , 1968) .

(III) Tissue samples of 50 g. from liver, kidney and muscles were used for detection and estimation of aflatoxin by thin layer chromatography (TLC) according to AOAC (1990) and High performance liquid chromatography (Waters HPLC) fluorescence detector , EX 338 nm^2, Emm 420 nm. and the mobile phase 70 (15 ACN + 85 H_2O) + 30 methanol 100 % according to Shepherd (1986) and AOAC (1995).

Tissues For Histopathological studies specimens from all visceral organs

and brain of a dead camel were preserved in 10 % neutral formalin. Paraffin tissue section from different organs were stained by H & E as a routine stain, Mason's trichrome stain for collagen fibers and Prussian blue for haemosidrosis . These stains were done according to the methods mentioned by (Clayden , 1971) .

Obtained data were analysed according to snedecor and Cochran (1967).

RESULTS

The clinical examination of the affected animals revealed the presence of nervous disorders mainly in the form of incoordination movement, paralytic like signs in the hind limbs and loss of reflexes. The body temperature in all cases was normal, ruminal stasis, some animals had tympany. The course of the clinical symptoms ranged from 3 to 4 days then the animal lie down and get into complete coma ended by death. The mortality rate reached 33% .

The results of isolation and identification of fungal species from the offered food revealed presence of toxogenic *Aspergillus flavus* together with *Mucor spp.* in all tested samples. High concentrations of aflatoxins were estimated in both green alfalfa and hay (Table I)

The results of biochemical analysis of serum as shown in Table 2 indicate significant increase in alkaline phosphatase (ALP), Alanine and Aspartate amino trasferases (ALT & AST) activities. While significant decrease were recorded in both urea and creatinine. The serum total proteins and uric acid levels were insignificantly decreased.

Examination of liver tissue for the presence of aflatoxins revealed the presence of all aflatoxins metabolites as showen in Table 3.

Gross pathological lesions:

Echymotic and petechial heamorrhages in subcutaneous fat and internal organs. The liver was enlarged, congested, friable and yellowish in colour. Spleen, omasum, abomasum, intestine, kidneys and urinary bladder were congested, the meningeal blood vessles of the brain were also congested..

Histopathological examination:-

The liver showed dilatation and congestion of the central veins as well as haemorrhages inbetween the hepatic cords. The hepatocytes appeared swollen with different stages of degeneration and necrosis as well as

vacuoles of different sizes (Fig 1). The portal area showed increased fibrous proliferation (Fig 2). Most of the intrahepatic bile ducts showed hyperplastic proliferation of its epithelial lining with the appearance of newly formed bile ductules.

The omasum, abomasum and intestine showed congestion and heamorrhages in the mucosa and submucosa, sloughing of the epithelial lining villi and infiltration by mononuclear cells (Fig 3). The spleen showed congestion and golden brown prussion, blue of hemosiderin pigment (Fig 4). The kidneys showed congestion of glomerular capillaries with expansion of mesangial space of some glomeruli (Fig 5) . Some renal tubules showed hyperblastic proliferation of the epithelial lining. The heart showed congestion and myocardial degeneration (Fig 6). The brain showed congestion of the meningeal blood vesseles and haemorrhages of variable degrees scattered in the white matter of the cerebrum. The wall of some blood vesseles was thickened and showed hyalinization (Fig 7). These changes were associated with perivascular, pericellular oedema, neurophagia and satellitosis (Fig 8).

Table 1: Aflatoxin estimated and fungi stains isolated from ration samples

Ration	Mucor Sp.	A. Flavus	A. Niger	Penicillin	Aflatoxin B_1
Green alfalfa	+ ve	+ ve	+ ve	- ve	100 ppb
Hay	+ ve	+ ve	- ve	+ ve	100 ppb
Wheat straw	+ ve	+ ve	- ve	- ve	20 ppb

Table 2: Serum total protein, urea, creatinine, uric acid levels and ALT, AST & AP activities in normal camels and those affected with acute aflatoxicosis.

Parameter	Normal Camels	Camels affected with aflatoxin
Total Proteins g/dl	6.35 ± 0.08	5.44 ± 0.23
ALT IU/L	9.0 ± 2	45.66 ± 1.91 ***
AST IU/L	78.0 ± 2	160.0 ± 3.2 ***
AP U/L	94.0 ± 18	544 ± 39 ***
Urea mgldl	12.2 ± 1.07	2.53 ± 0.23 **
Creatinine mg/dl	1.42 ± 0.07	0.51 ± 0.08 **
Uric acid mg/dl	4.0 ± 0.25	3.08 ± 0.29

** significant at $p \leq 0.01$ *** significant at $p \leq 0.001$

Table 3 : Estimated values of Aflatoxin in the liver tissues

Aflatoxins	Concenteration
G_1	83.07 ppb
B_1	66.7 ppb
G_2	11.3 ppb
B_2	33.6 ppb

DISCUSSION

Aflatoxins are potent liver toxins, and their effects in animals vary with dose, length of exposure, species, breed and diet or nutritional status. These toxins may be lethal when consumed in large doses, sublethal doses produce chronic toxicity and low levels of exposure can result in cancer (Sinnhuber et. al., 1977). Aflatoxicosis has been reported in most countries, in many spoiled feeds, and mainly on harvested crops rarely on a standing crops.

In the present study the isolation of highly toxogenic *Aspergillus flavus* strains and detection of their toxins from the green alfalfa, hay and wheat straw offered to camels were incriminated as a causative agent of illness and deaths among camels because of negative results of viral, bacterial and parasitical examinations. In the present study the detected amount of aflatoxin in green alfalfa and hay was 100 p.p.b. for each. This concenteration exceeded the maximum tolerated levels of mycotoxins in animals feed stuffs in Egypt according to Egyptian standard specifications 1875 - 1990 which is 10 - 20 p.p.b.

Similar clinical findings of aflatoxicosis in camels were reported in Al Ain area in United Arab Emirates by Osman & Abd El Gadir (1991) and Samy et. al. (1993), where they found loss of appetite, dullness, loss of reflexes, ruminal stasis, paralytic like signs in the hind limbs and finally death. The possibility of the exposure of camels in Egypt to aflatoxin was found where Balata and Bahout (1996) detected aflatoxin M_1, in 25 % of camels milk collected from the north wastern coastal desert of Egypt.

Contamination of green food by the fungal toxins while in the pasture was previously recorded by Mansfeld et al. (1989), Alessi et. al. (1994). Scruggs and Blue (1994) attributed the presence of fungal toxins in the alfalfa hay to prolonged periods of wet weather that caused delayed cutting and mold growth perior to harvesting.

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Regarding the biochemical serum analysis the noticed decrease in total proteins may be attributed to the impaired protein synthesis as a result of liver affection due to aflatoxicosis (Edds, 1973). These findings were in consistence with that recorded by Mert et al. (1988) and Beers et. al. (1992). The increase in ALT and AST activities were previously reported by Edds (1973) and Zuric & Stankovic (1991) in Pigs and Beers et al. (1992) in fowl due to liver affection in case of aflatoxicosis. The elevation in ALP activity comes in consistence with that mentioned by Jassar and Balwant (1993) in chicken and Harvey et al. (1995) in lambs who suggested that this was due to degenerative changes in the liver causing leakage of the enzymes into serum.In the camels Afzal and saeed (1995) reported that the highest concentration of alkaline phosphatase presents in the intestine may explain the great increase in the alkaline phosphatase activity due to damage of intestinal wall together with liver damage. Also there is a significant decrease in serum urea , similar findings were reported by Edds (1973) in young pigs given toxic amounts of aflatoxin. The non significant decrease in uric acid is in agreement with that reported by Mert et al. (1988). The concomitant decrease in total proteins, urea , creatinine and uric acid with the increase in the estimated enzymes are indicative to liver insufficiency. The detection of aflatoxins in the liver tissues is due to that liver is the primary site of metabolism of ingested aflatoxins as well as the primary location of residues and lesions (Heathcote and Hibbert, 1978). Regarding the gross pathological lesions, simillar findings were reported by Edds (1973) in sheep and Samy et al . (1993) in camels.

Microscopically, bile duct hyperplasia in the liver as shown in the present study was reported in pigs following short term oral dosing with lower levels of crude aflatoxins (Sisk et al., 1968). The detection of the newly formed bile ductales was similar to that reported by Kelly (1985) who suggested that the hyperplasia of the bile ductes is an attempt to regenerate hepatic parenchyma when the parenchymal cells have lost their capacity to regenerate themselves. The presence of golden brown Prussian blue positive haemosiderin pigments may be attributed to the excess hemolytic activity which occure due to toxin (Jones and Hunt, 1983). Similarly kidney lesions were reported with mouldy corn poisoning and experimental aflatoxicosis in pigs (Sippel et al., 1953 and Sisk et al., 1968). Generally the histopathologic changes in aflatoxicosis varied according to the period of exposure and the

concentration of aflatoxin to which the animals has been exposed (Newberne, 1973).

In conclusion, the present study showed that the liver is the primary site of metabolism of ingested aflatoxin as well as the primary location of residues and lesions. The metabolism of aflatoxin result in the alteration of various metabolic processes within hepatocytes which leads to severe serum biochemical alterations and serious pathological changes.

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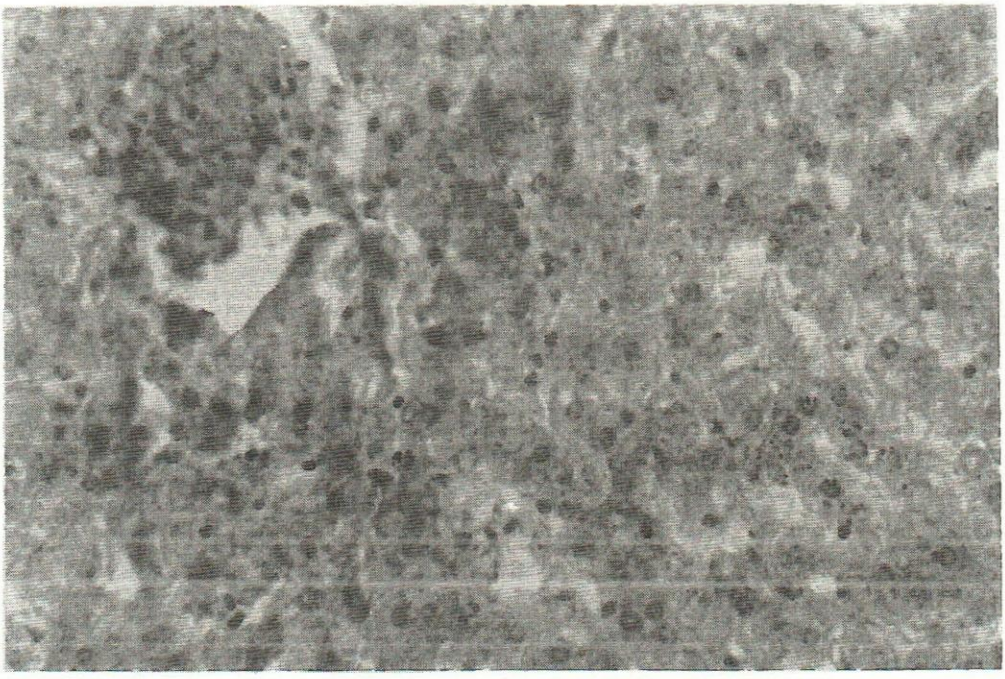

Fig (1) Liver showing different stage of degeneration, necrosis and vacuoles of variable size (H & E X 40)

Fig (2) Liver showing bile duct proliferation and necrosis associated with fibrosis (Masson's trichrom stain , X 40)

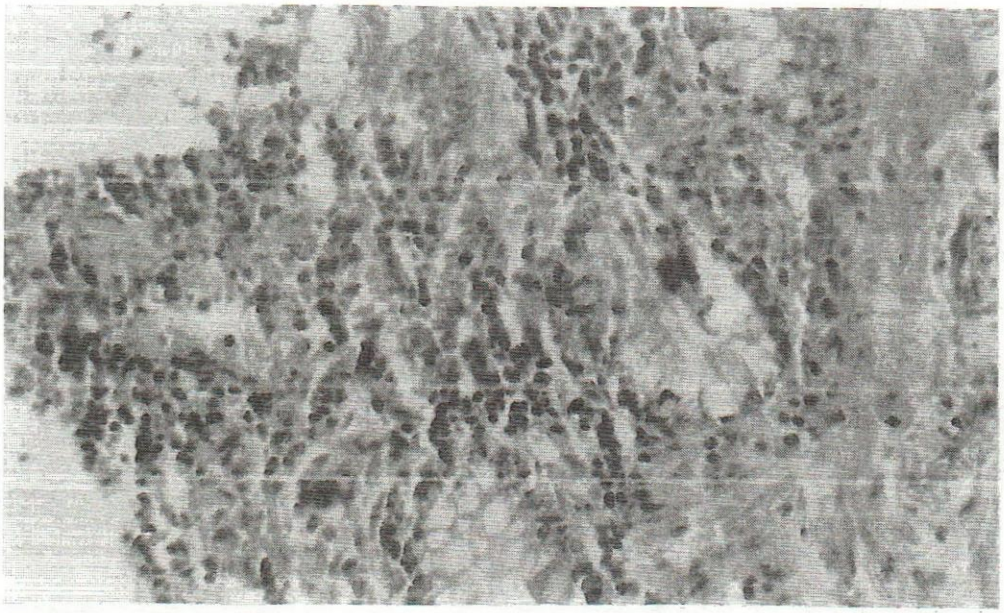

Fig (3) Intestine showing congestion in mucosa and submucosa, sluffing of epithelium lining, haemorrhage and infilatration with mononuclear cells (H & E X 20)

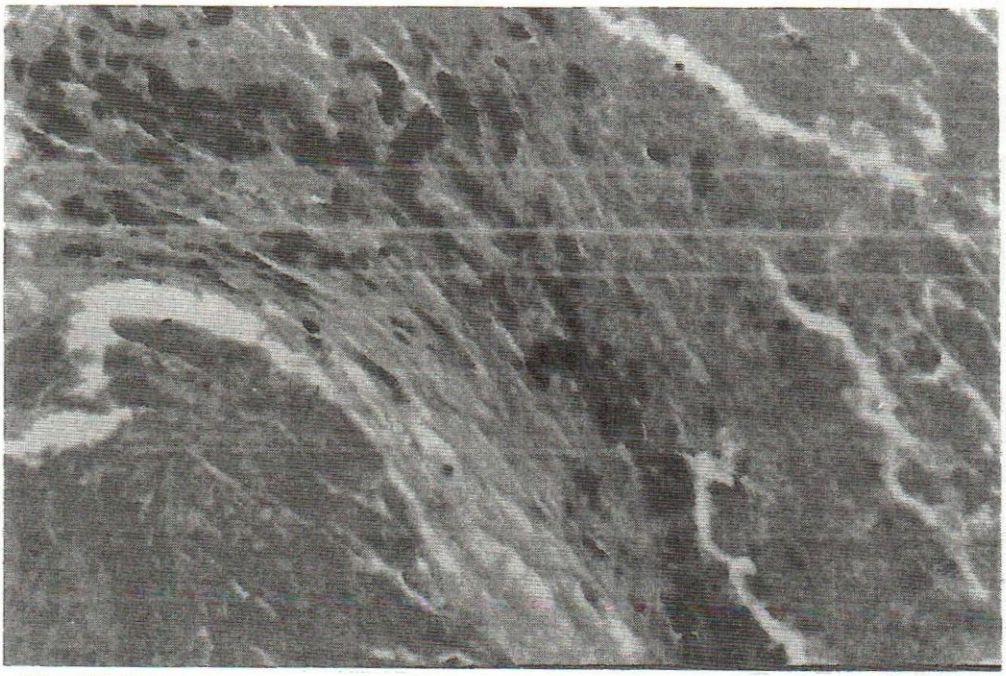

Fig (4) Spleen showing Hamosidrosis (Prussian blue stain X 20)

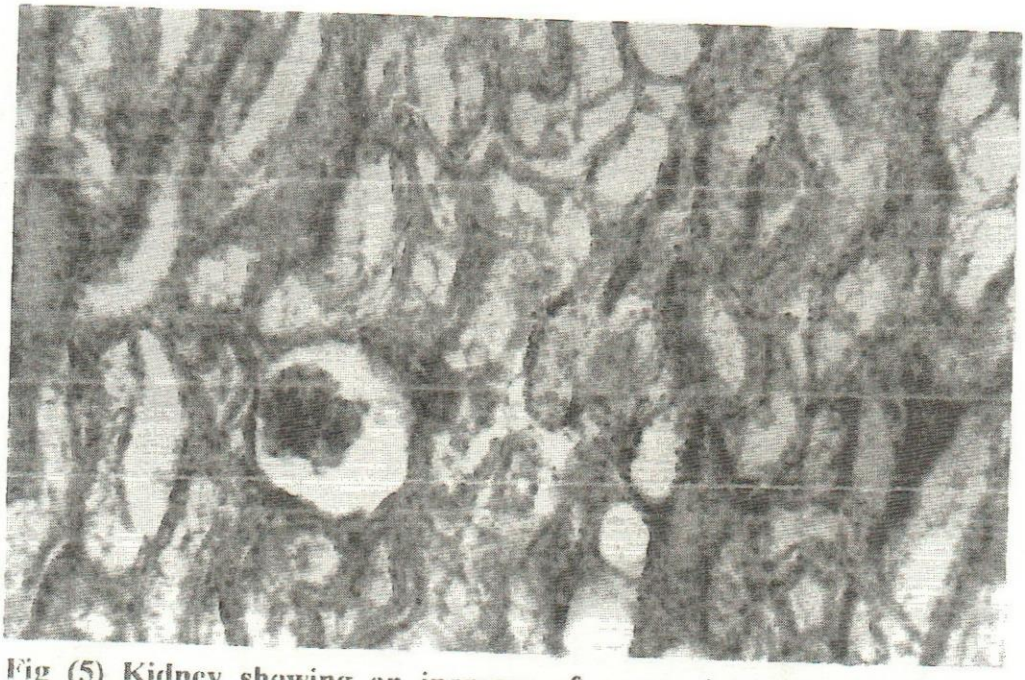

Fig (5) Kidney showing an increase of mesangial space with hyper proliferation of epithelial lining of renal tubules (H & E X 40)

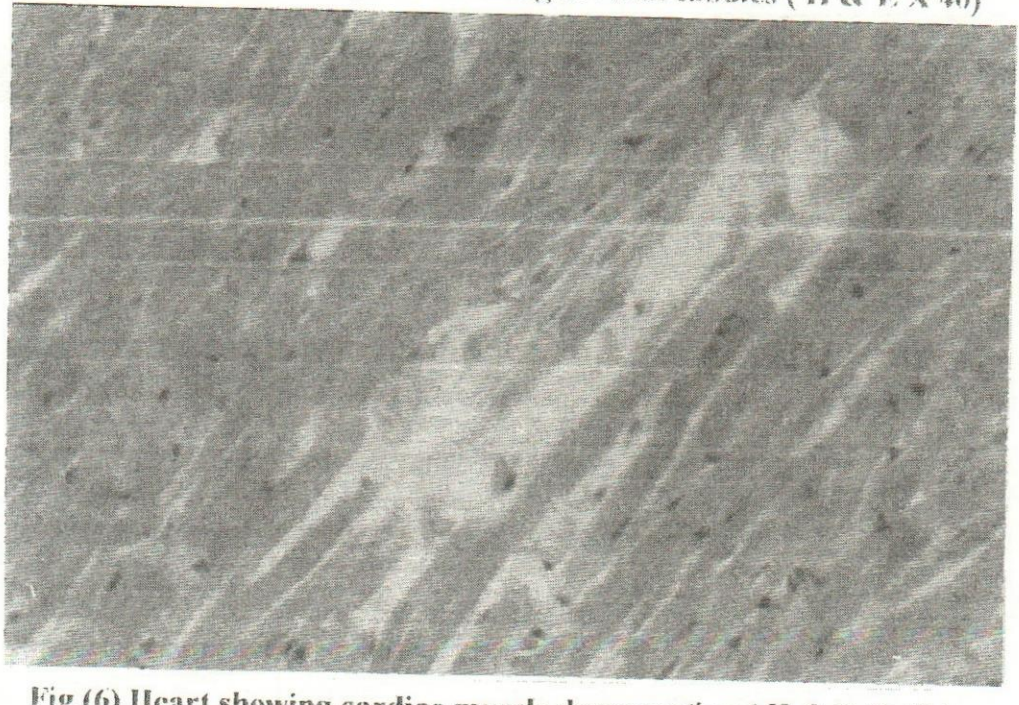

Fig (6) Heart showing cardiac muscle degeneration (H & E X 40)

Fig (7) Brain showing hyalinization of blood vessels (H & E X 40)

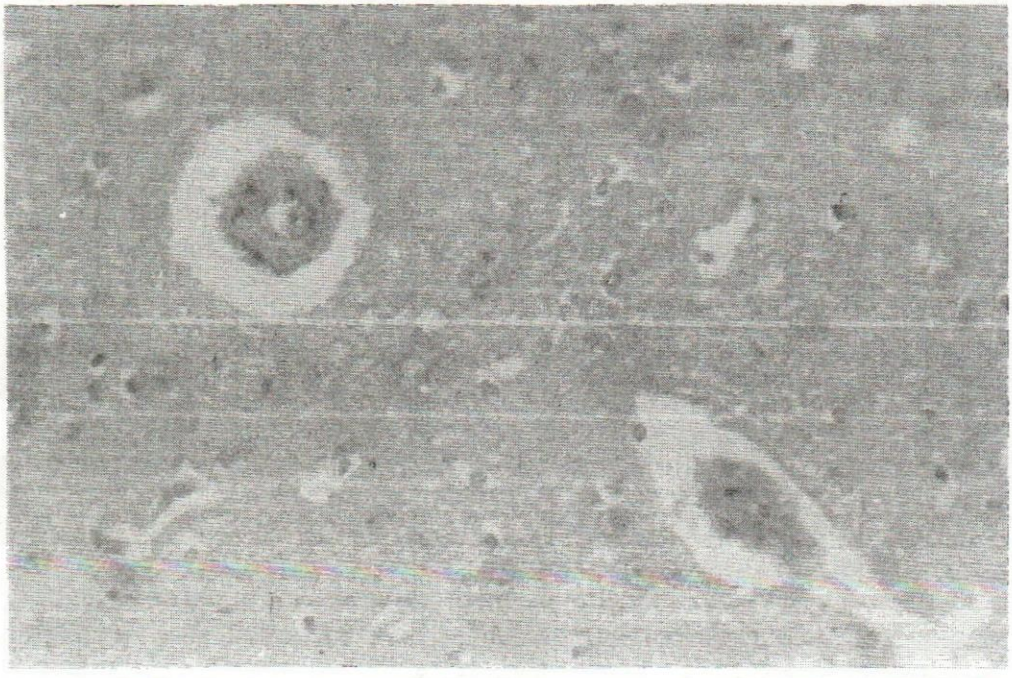

Fig (8) Brain showing perivascular , pericellular Odema, satelletosis and neurophagia (H & E X 40)