

Study of unusual clinical cases in Iraqi Farm Animals

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Abstract

During a period extended in between 1998 into 2009 years sixth unusual clinical cases, were register. These cases were brought to private clinic and clinic of Veterinary Medicine College, which includes the followings: large subcutaneous cyst located at the neck of a buffalo aged eight year were presented. The cyst developed as a small swelling and gradual increase in size in a period of 5-6 months. The cyst was treated surgically by complete evacuation, and packed with drain. The second cases, four year female buffalo, having a tissue mass above the carpal joint. This mass was a gradual increase in size, after parturition. The content was aspirated; the physical examination revealed that, this content is milk and the case as an ectopic mammary tissue. While the third cases, nine year old cross breed cow with history of having pregnant of seven month, showing decrease appetite, abnormal harsh cough and enlargement of larynx. After complete examination and investigation, there was sharp metallic needle inside the larynx..Laryngotomy was performed to remove of the foreign body. After fourteen days of operation, the animal returns to normal condition.. The four case, eight-year old cow with a history of having seven month pregnant was delivered dead large fetus with two heads and two necks. The post mortems revealed that the two necks connected at the first thoracic vertebrae and there was anasarca and ascitis. The last two cases represented by ameloblastoma with a history of mass close to the mandible of old local breed sheep was diagnostic. Ameloblastoma is present as a swelling and appear radiographically as radiolucences lesion and histopathologically composed of unilocular or multilocular cyst and cords of epithelium. It treated by complete lesion excision. And, the last one a case of osteoblastoma arising in the frontal bone of local breed sheep 5 year in aged was diagnostic. It present as swelling and appear radiographically as radio-opaque lesion, the histopathological is of a highly vascularized stroma with immature bone. This lesion is treated by complete excision.

دراسة لحالات سريرية نادرة في الحقول الحيوانية في العراق

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الخلاصة

تم تسجيل ست حالات نادرة في العيادة الخاصة وكلية الطب البيطري خلال الفترة بين 1998-2009. الحالة الأولى وجود كيس كبير تحت الجلد في منطقة الرقبة لجاموسة عمرها 8 سنوات حيث بدأ صغيراً ثم بلغ حجماً كبيراً خلال 5-6 أشهر وتم علاجه بتفريغ السوائل ووضع فتيلة. أما الحالة الثانية فوجد ورم صغير فوق مفصل الركبة وازداد في الحجم بعد الولادة و إثناء سحب جزء من محتوياته وفحصه تبين انه حليب وشخصت الحالة بأنها نسيج من الضرع . أما الحالة الثالثة فكانت لبقرة عمرها 9 سنوات وحامل 7 أشهر لوحظ عليها فقدان بالشهية وتعاني من سعال خشن مع تضخم الحنجرة وبعد الفحص تبين وجود جسم معدني داخل الحنجرة وتم استخراجه بعملية فتح الحنجرة وقد عادت البقرة إلى الحالة الطبيعية بعد 14 يوماً من العملية. وإما الحالة الرابعة لبقرة عمرها

حوالي 8 سنوات وقد ولدت عجلا بعد 7 أشهر تقريبا من الحمل برأسين ورقبتين وعند عمل الصفة التشريحية وجد تمفصل الرقبتين مع الفقرة الصدرية الأولى مع وجود استسقاء وحبن. أما الحالتين الأخيرتين تمثلت إحداهما بوجود ورم أرومة مينائية في الفك الأسفل لنعجة وأظهرت الصورة الشعاعية وجود منطقة متعظمة بيضاء ومنطقة فاتحة وأظهرت الصفة النسجية المرضية فجوات أحادية و متعددة مع بروز على شكل عمود من الظهارية وعولجت باستئصال الورم. وأما الحالة الأخيرة فوجد ورم أرومة عظمية فوق عظم الجبهة في نعجة عمرها 5 سنوات وظهر الفحص الشعاعي وجود آفة متعظمة فاتحة اللون، أظهرت الصفة النسجية المرضية وجود عظم غير ناضج وعولجت باستئصال الورم.

In buffaloes:

Large cyst of subcutaneous Tissue.

Introduction

Cysts are any closed cavity or sac, normal or abnormal lined by epithelium and especially one that contain a liquid or semiliquid material. The ganglion cysts were diagnosed in a 4 month old male Afghan Hound. The subcutaneous ovoid cysts is located around the caudal right elbow joint and left ischiatic tuberosity and had abundant mucinous fluid and internal folding. The lesions recurred twice around the elbow joint after surgical removed(1). The other workers(2) describe that, the mean long diameter of the epidermal inclusion cyst in 13 patients were 3 cm. The common sites of this cysts were the planter surface of the metatarsal phalangeal joint and buttocks. Other workers (3), findings that intralesional formalin administration for treatment of subepiglottic cysts, in four year old horse may be a minimally invasive, economically suitable alternative to surgical treatments.

Materials and Methods

Buffalo aged eight years developed small swelling in a middle of right side of the neck and it was gradual increase in size during a period of 5-6 months. The swelling fluctuating was occupied the area between the ear and prescapular region (Fig.1 a and b). Upon palpation of the tissue mass it became evident that they were in a subcutaneous tissue and the presence of clear fluid was confirmed by exploratory puncture, indicated that there is a cyst. The animals was sedated by xylazine hydrochloride at a dose 0.05 mg/kg.B.W intravenously, then under aseptic surgical technique, content was aspirated from the soft distal point of the swelling by using needle (G 18). Incision about 1 cm was made in the same site of aspiration to provide a complete evacuation of the content. The amount of aspirated fluid for about 4-4.5 liters The cavity was injected with 1% copper sulphate and repeated after 3 days. Then after that it's treated as an abscess. The opening was wide and washing by iodine and then packed by drain (gauze soaked with tincture iodine), and exchange every 48 hours until obliteration of cavity. Treatment was continued (1.5 month) to complete obliteration of cavity and retain to apparently normal after a period of about 3 months (Fig. 1, c).

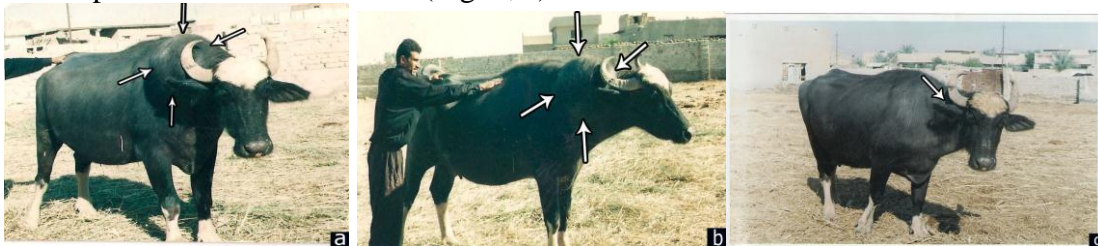


Fig. (1) Large cyst on the right side of the neck in buffalo before treatment. a, (front view)(arrow \Rightarrow), b (Lateral view) (arrow \Rightarrow), and c, 3 months after treatment. (arrow \Rightarrow)

Discussion

The subcutaneous swelling diagnostic in this study was a cyst according to palpation and appearance of fluid. It is treated by evacuation and injection with 1% copper sulphate but this substance may be insufficient to destroy the epithelial cells which excreted fluid, as well as large cavity of cyst. The cavity was contaminated by micro-organism so that, discharge turbid fluid exteriorized similar to abscess. Prolong using of drain, act as antimicrobial and stimulation of granulation tissue to obliteration of cavity. This procedure was required long time (1.5 months); this may be due to the large cavity, which needed long time to obliterate by granulation tissue. While (3), reported that the subepiglottic cyst (2cm in diameter) in a horse was treated with intralesional formalin administration following two injection, two weeks apart, the cyst was completely resolved with no evidence scarring or epiglottis deformity. The review of available literature, become evident that the large subcutaneous cyst is rare reported.

Ectopic mammary Tissue

Introduction

The mammary glands are derived from the sweat glands and that they are derived embryologically by the invagination of ectodermic buds into underlying mesoderm (4), (5), and (6). Ectopic mammary tissue has been reported in the wall of the teat canal in cows (4). Also ectopic mammary tissue of vulva was recorded in ten goats belonging to the Nubian and Syrian breeds (7).

Materials and Methods

Four years old female buffalo was referred to our private clinic with a history of having tissue mass above the carpal joint of right fore limb. The animal was of a normal condition. After parturition, there was a gradual increase in the size of the mass. Physical examination indicated subcutaneous tissue may be of the tissue mass. Aspiration of the mass under aseptic technique using a sterile syringe appeared that fluid white in color closely resembling milk in smell and consistency. This fluid was boiled and taste. Physical examination of the white fluid as well as tasting, indicated that, this fluid is milk. The mass diagnosed as a ectopic mammary tissue above the anterior aspect of carpal joint of the right fore limb in buffalo.

Discussion

The tissue mass reported in this study was ectopic mammary tissue as indicated by clinical and physical examination of white fluid obtained through aspiration from subcutaneous mass. This observation was supported by the owner, claimed that the mass was gradual increase in the size following parturition. This observation coincided with other worker in goats (7) whom they said that diagnosis ectopic mammary tissue in vulva of goats. Ectopic mammary tissue over the cranial aspect of carpal joint in buffalo was perhaps unavailable in literature.

In cow: Sharp metallic foreign body on the larynx

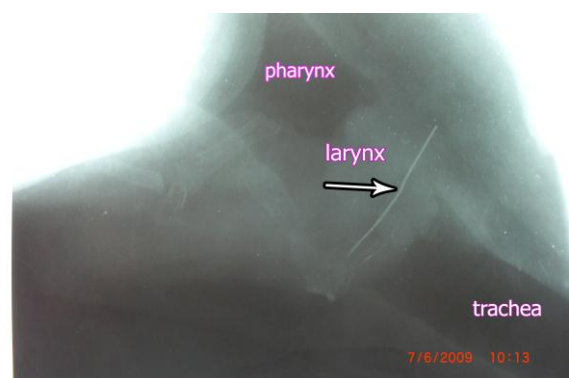
Introduction

An unusual presentation of a firework penetration injury resulting in a sharp coiled metal foreign body traveling through a small entry wound in the neck and subsequently lodging itself in the tracheobronchial tree (8). Also (9) show the first bones has been reported to cause upper respiratory airway tract abscesses. However the migration through the entire pharyngeal wall ending in a superficial cervical abscess (10). Their removal is essential to prevent super infection, abscesses and perforation (11).

Materials and Methods

Nine year old cross breed cow, with a history of having pregnant of seven months. The clinical signs was a decrease in appetite, abnormal harsh cough, and received injection of systemic antibiotic for four days which repeated twice again without results. Physical examination revealed enlargement of larynx with swelling of surrounding tissue. The examination of the site by metal detector investigate presence of metallic foreign body, beside that radiography of the larynx to confirm the diagnosis (Fig. 2). The animal sedated by xylazine hydrochloride at a dose 0.05 mg/ kg B.W intramuscular. Local anesthesia at the site of operation is used. Laryngeotomy was performed to remove the foreign body. Under aseptic technique ventral mid-line cervical incision is done directly over the larynx in order to open thyrocricoid ligament. After reflexion of muscles laterally, small incision was induce in ligament then enter the finger inside lumen of larynx to detect the foreign body and removed by curved artery forceps which introduced and grasping metallic foreign body. The incision around opening in ligament was sutured as a routine manner, while the incision of ligament leaves without suture for spontaneous healing. Pencilliin-stresptounycin at a dose of 10,000 I.U,20mg/kg B.W intramuscular respectively was done for four days post operation. A skin suture was removed after 10 days post operation. Fourteen days later, the animal was in a good condition and the site of operation was free from complications beside that the incision of ligament was healed.

Fig. (2) Sharp metallic foreign body inside the larynx (arrow \Rightarrow)



Discussion

Usually metallic foreign bodies were interred during swallow of food mainly into reticulum, but in a rare cases the sharp foreign bodies penetrated the mucosa of mouth, pharynx or esophagus which lodged within its or passes to the surrounding tissue with the subsequent cellulites or abscess formation. The presented case, this sharp foreign body within the bolus penetrated the ventral mucosal layer of esophagus at the level of dorsal aspect of larynx, and lodged in middle lumen of larynx. This was indicated by the direction of foreign body

radiographically. This observation coincided with (8), who said that sharp foreign body penetrated neck and logon in tracheobronchial tree. The enlargement of larynx and surrounding tissue may be due to contamination of foreign body, lead to inflammation of lumen of larynx and surrounding tissue. The subsequent of this inflammation caused abnormal harsh sound with the systemic reaction and decrease appetite. So that large doses of systemic antibiotic for along period without response. While after removal of source of infection and used systemic antibiotic gives good results. Altimately after fourteen days of operation the animals appeared in a good condition. This can be considered as a rare case.

A congenital abnormality of the calf

Introduction

The authors (12) describe that a 10 days old male Holstein dairy calf with orthopedic abnormalities was unable to stand but was alert with a suckle reflex. At necropsy, the calf showed multiple defects, including partial agenesis of the left rib plate, deformed left scapula, shortened left humerus, agenesis of the left kidney, artesia ani and scoliosis .While (13) showed that polydactyl in shami breed goat. At birth a male goat had an increase in the number of both hind leg digits. It has been concluded that polydactyl in goats is heritable. Other workers (14), mention that the outbreak of congenital chondrodysplasia in calves in south east Australia with no specific etiology could be determined. There is some evident that the cause of the deformities could be a manganese deficiency during fetal development. Also (15) showing lateral anophthalmia and deformation of the jaw such as brachygnathia superior and bilateral cleft.

Material and Methods

Eight-year old brown and white cow was referred to private clinic with a history of having, pregnant of seven month and signs of parturition was observed such as vaginal discharge, straining and restlessness. Examination and doing assessment for delivery because the fetus large in size, but exteriorized without cesarean section. Brown dead calf was aborted with two fully formed heads and two necks connected to the body just at the thoracic inlet, with first thoracic vertebrae, very large abdomen with anasarca and ascitis also seen (Fig.3).

Fig. (3) Two heads, two necks of seven months old calf



Discussion

Animals born with two head, a condition known as polycephaly were register, while in this study a calf abortion with two heads and two necks was registred. The necks were connected at the thoracic inlet with first thoracic vertebrae. In this study the etiology was not determined due to the owner who is not mentioned sufficient information about the cow. In addition to that, unable to detection the antibody of several viral diseases, which may interfere with a congenital abnormality of pregnant infected cows. In literature, they reported that several factors may play an important role in this field. The Alkabane virus is the etiological agent of epizootic abortion and congenital arthogryposis. Besides hydranecephaly syndrome in cattle was noticed (16). Also (17) whom said that Akabane disease, an infections disorder causing congenital abnormalitie in calves. Beside that (18), describe that stillborn male calf from an embryo transfer recipient was carried out. Two normal heads were present on two necks which were fused at the shoulder is register in this study which believable that followed with the hypothesis due to atypical hatching, that is emergence of the blastocyst from the zona pellucida, may cause anomalous twinning.

In sheep: Ameloblastoma of the Mandible

Introduction

Ameloblastoma mean amel (enamel) and blastos (germ) is a rare benign tumor of odontogenic epithelium much more commonly appearing in the mandible than the maxilla . Authors (19), mention that the ameloblastoma is the slow growing benign tumor of the jaw and patients usually present late after the tumor achieved considerable size to cause facial disfigurement. Other workers (20), reported that, male llama was presented because of rapidly enlarging mass on the right of the face. The mass did not interfere with mastication and did not appear the painful. Radiographs revealed a marked expansion of the right caudal face region with bone lysis involving the maxilla, nasal, lacrimal, zygomatic and palatine bone. Histopathologically, the mass consisted of anastomosing cords and sheets of neoplastic odontogenic epithelium. The histological diagnosis was keratinizing ameloblastoma. On other hands ameloblastoma has also been identified in horse and cattle (21), (22), and in sheep (23).

Materials and Methods

Old local breed sheep with a history of having mass close to the mandible, this swelling was gradually increase in size at a period of about 4 month. The swelling was the result of an expansion of the cortical bone of the jaw and can be identified by palpation as hard and bony. It was associated with the difficult mastication and feeding. The radiography has been taken to evaluate the lesion and condition of adjacent structure. The animal was sedative by xylazine hydrochloride (0.05 mg/kg.B.W) intramuscularly and local anesthesia at the site of operation was applied. The site of operation was prepared for aseptic surgery. The tissue mass was bluntly dissection from the surrounding tissue. The incision was closed by routine manner. post operation care used penicillin- streptomycin 10000 I.U,20mg/kg B,W intramuscular respectively for four days. Biopsy was imbedded in 10% neutral buffered formalin for 72 hours, histopathological section was prepared routinely and stains by hematoxylin and eosin. The histopathological revealed that, there is a nest or cord of stratified squamous or columnar epithelium are embedded in a loose fibrous stroma. The tumor cells which are resemble ameloblastoma oriented perpendicular to basement membrane form bands that separate the tumor from stroma (Fig. 4,a). Hemorrhage and inflammatory cells infiltration, were seen in tumor stroma (Fig. 4,b). In other section there is a cystic variants, lining by stratified squamous or columnar epithelial cells which may be flat and regular or thrown, up into papillar projection(Fig.4,c).. Radiograph appeared as radiolucent lesion that may have either unilocular or multilocular appearance.Expansile bony mass was present at the right aspect of the mandible (Fig. 5).

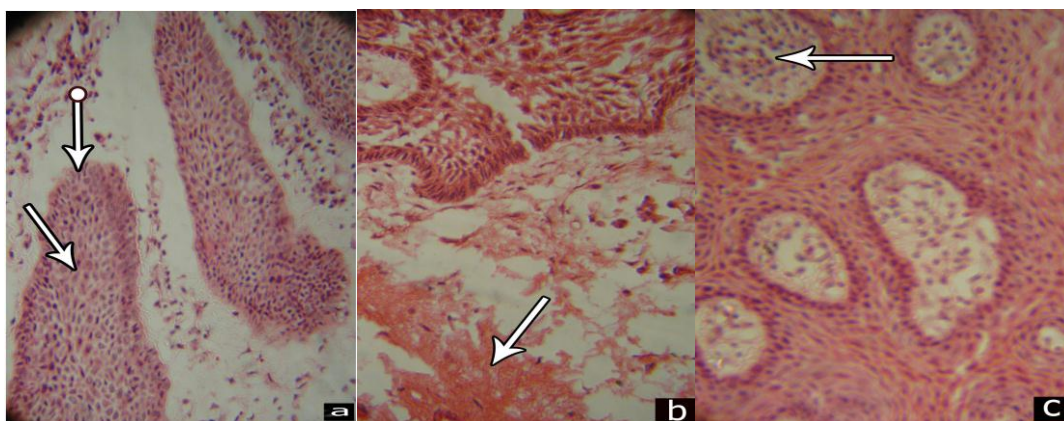


Fig. (4) Cord of stratified squamous or columnar epithelium (a) (arrow \Rightarrow), tumor cells perpendicular to basement membrane (a) (arrow \Rightarrow), hemorrhagic area (b) (arrow \Rightarrow), cyst (c). (arrow \Rightarrow)



Fig. (5) Ventro-dorsal (a), and lateral views (b) radiograph, show expansive bony mass of the right aspect of the mandible, and radiolucent lesion) (arrow \Rightarrow)

Discussion

Ameloblastoma is a benign tumor of bone especially mandible, but the tissue growth may be aggressive in the involved area. It can progress to great size and cause displacement of teeth, loose teeth, facial asymmetry, malocclusion and pathologic fracture (19). In our case, the history, clinical signs, physical examination, radiography along with the tissue biopsy was diagnosed as ameloblastoma of mandible. Complete surgical excision was done in order to prevent invasion of other adjacent structure in addition to prevent recurrence. This agreement with other workers (19) and (24) whom said that, treatment of choice is surgical excision with wide free margin. Other treatments such as radiation or chemotherapy were not successful for treatment of ameloblastoma (25).

Osteoblastoma of the frontal bone

Introduction

Osteoblastomas account for approximately 3, 5% of all benign primary bone tumor (26). Osteoblastomas located on the surface of cortical bone, or periosteal osteoblastomas are extremely rare. An unusual case of periosteal osteoblastoma located in the frontal cranial bone (27). Although osteoblastomas may affect virtually any bone but the most common site is the vertebrae (28). It usually located in the medullary cavity of the flat and long bones and periosteal location are rare (29). The radiation therapy or chemotherapy is controversial in the treatment of osteoblastoma. The treatment goal is complete surgical excision of the lesion (30).

Materials and Methods

Local breed sheep 5 years in aged was preferred to our clinic with history of having growth near the base of horn. The owner observed that the growth was gradual increase in size for about 2 month ago. This growth reached diameter of about 10 cm. Clinical signs and by palpation of the growth tissue appearance of hard tissue was diagnostic. Radiograph has been taken to detect the lesion. It present as swelling and appear radiographically as radio-opaque, radiolucencies to poorly defined mixed lesion (Fig. 6). The site of operation was prepared aseptically. Under deep sedation of xylazine hydrochloride at a dose 0, 05 mg/B.W intramuscular and local anesthesia around the mass and blunt dissection was used to complete excision of growth; incision was closed as routine technique. Post operation penicillin streptomycin at a dose of 10,000 I.U, 20mg/kg B.W intramuscular respectively was used for four days. Ten days later, the sutures of skin were removed. The tissue biopsy was fixed in 10% neutral buffered formalin for 72 hours. Histopathologic section was prepared routinely and stains with hematoxylin and eosin. The histopathological revealed that, there is randomly inter-connecting trabeculae of woven bone that are prominently rimmed by osteoblast. The stroma surrounded the tumor bone is loose connective tissue that contain many dilated and congested capillaries(Fig.7,a). Osteoclast are present in fiber vascular connective tissue(Fig.7,b) Immature fibrous connective tissue with irregular direction and pleomorphic cells are also reported (Fig.7,c). Cartilage tissue was also seen.



Fig. (6) Lateral view of sheep skull, show radio-opaque, closed to the frontal bone. (arrow ⇒)

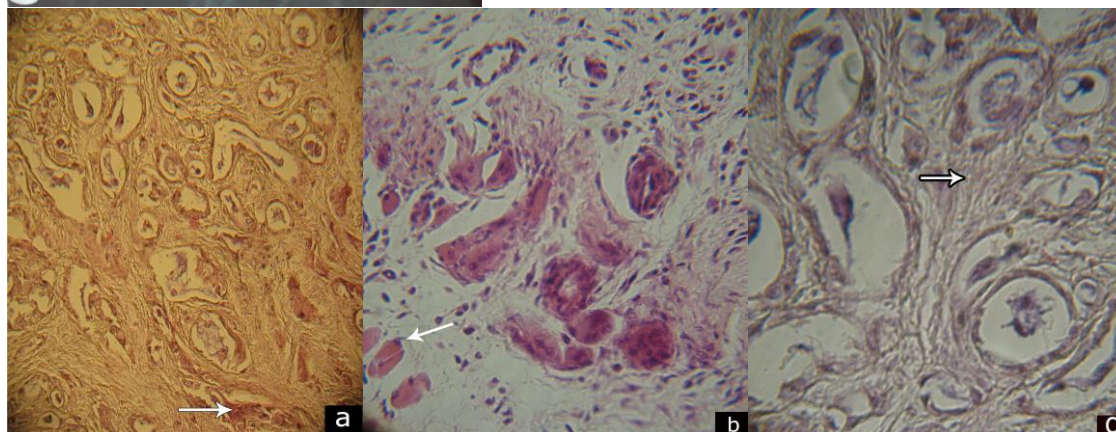


Fig. (7) Congested blood vessel (a), osteoclast (b), immature fibrous connective tissue with irregular direction and pleomorphic cells (c)

Discussion

Most of authors referred that osteoblastoma are most commonly found in the vertebral column, followed by the long bones, in particular the femur and tibia, and they are much less common in other bones (26), (31). While in this study the osteoblastoma found in the skull, which indicated by notices mention above. Complete surgical excision of all growth was exhibited good result. This observation coincides with other workers (27) whom said that the response of benign osteoblastoma to conservative surgical treatment is generally good.

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Serological study of Leptospirosis in cattle, sheep and goats in Baghdad Province.

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Abstract

A total of 565 serum samples from cattle (260) sheep (171) and goats (134) were screened for the presence of Leptospiral antibodies by macroscopic plate agglutination test and microscopic agglutination test. The prevalence was found to be 57.3% in cattle, 24.6% in sheep and 22.4% in goats. *L. hardjo*, *L. mini*, and *L. hebdomadis* were found to be more common by using microscopic agglutination test.

دراسة سيروولوجية لداء البريميات في الأبقار والأغنام والماعز في محافظة بغداد

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كلية الطب البيطري/ جامعة بغداد

الخلاصة

لقد تم فحص (565) عينة مصلية شكلت الأبقار (260) عينة والأغنام (171) والماعز (134) وذلك للكشف عن وجود الأجسام المضادة باستعمال اختبار الصفيحة العياني واختبار التلازن المجهرى حيث وجدت بنسبة 57.3% في الأبقار و 24.6% في الأغنام و 22.4% في الماعز. ووجدت الأنماط المصلية *L. hardjo* و *L. mini* و *L. hebdomadis* الأكثر شيوعاً باستعمال اختبار التلازن المجهرى.

Introduction

Leptospirosis is an infectious disease of man and animals occurs in all farm animals species and is an important zoonotic diseases caused by the members of genus leptospira. The disease is universal in distribution and causes sever losses in domestic animals, particularly in cattle (1) (2) (3). Human, animals or environment that may be contaminated by infected animals (4) (5). Convalescent and chronic cases act as carriers by the leptospira in urine for a considerable length of time (6). It costs farmers considerable amounts of money every year due to losses resulting from abortion, fetal death, stillbirth, repeat breeding , production failure and reduction of milk (7) (8).

Review of literature revealed that there is no published report about its prevalence in Iraq. Therefore the purpose of this study was to conduct a serological survey on serum samples from cattle, sheep and goats from different areas in and around Baghdad province to determine the prevalence of leptospirosis in sera of them.

Materials and Methods

A total of 565 serum samples were collected from cattle (260), sheep (171) and goats (134) of different ages, breeds, and localities in and around Baghdad. Majority of these animals were clinically normal, while some had a history of mastitis, abortion, retained placenta or infertility.

- **Serological technique:**

Macroscopic plate agglutination test was used in this study. This test has been developed by Galton (1) in which formalin killed organism was used as antigen.

Microscopic agglutination test, this test has been employed in this study as shown in (9) (10) (11).

- **Antigens:**

Pool and individual leptospira antigens were obtained from Difco laboratories*.

Serum samples from 74 cow, 28 sheep and 19 goats were send to WHO/ FAO collaborating center for reference and research on leptospirosis, Royal tropical institute in Amsterdam employing microscopic agglutination test with live antigen using 24 serotype for further screen. In addition, serum samples from 75 cow, 29 sheep and 19 goats were sent to WHO/FAO reference laboratories for leptospirosis at public health laboratory service, county hospital, Herford United Kingdom for microscopic agglutination test with formalized antigens which they have been used 19 serogroups.

Results

A total of 565 serum samples were collected from cattle, sheep and goats as shown in Table (1). The results of total positive samples by using macroscopic plate agglutination test and prevalence for each species are summarized in Table (2), while in Table (3), showed the results of positive serum samples with individual leptospira antigens.

Table (1) Source of serum samples

| Source | Cattle | Sheep | Goats |
|-----------------------------|--------|-------|-------|
| Baghdad Slaughter house | 55 | 140 | 103 |
| Different Farms | 37 | 17 | 16 |
| Al-Faudailia | 74 | 9 | 0 |
| Al-thahab Al-Aabyad village | 70 | 2 | 8 |
| Clinic in Baghdad | 24 | 3 | 7 |
| Total serum samples | 260 | 171 | 134 |

Table (2) Results of Macroscopic Plate Agglutination Test of serum samples with pool* and individual Leptospira antigens

| Animal species | Total examined | Total positive | Prevalence% |
|----------------|----------------|----------------|-------------|
| Cattle | 260 | 149 | 57.3 |
| Sheep | 171 | 42 | 24.6 |
| Goats | 134 | 30 | 22.4 |

Table (3) Results of Macroscopic Plate Agglutination Test of serum samples with individual Leptospira antigens

| Leptospira antigens | Cattle | | Sheep | | Goats | |
|---------------------|-------------------|-------|-------------------|-------|-------------------|-------|
| | Total N0. exam./+ | +ve % | Total N0. exam./+ | +ve % | Total N0. exam./+ | +ve % |
| icterohaemorrhagiae | 260/28 | 10.8 | 171/7 | 4.1 | 134/6 | 4.5 |
| grippotyphosa | 260/13 | 5.0 | 171/0 | 0 | 134/5 | 3.07 |
| pomona | 124/9 | 7.3 | 39/1 | 2.6 | 32/2 | 6.3 |
| hardjo | 260/78 | 30 | 171/12 | 7.0 | 134/11 | 8.2 |

Out of 74 cattle serum samples examined with live antigens, 63 samples reacted positively with one or more leptospira antigens when tested against 24 strains representing different serotypes. Twelve out of 28 sheep and 11 out of 19 goats showed

*DIFCO Laboratories, West Molesey Surrey U.K.

positive reaction with similar test as observed in table (4). All sheep and goats sera reacted at low dilution 1:20, 1:40. They failed to react at dilution 1:80 or higher.

Table (4) Results of microscopic Agglutination Test– Live Antigen– on Cattle, Sheep, and Goats serum samples at Royal Tropical Institute- Amsterdam

| Animal Species | No. exam. | No. positive | Serum Dilution | | | | | | |
|----------------|-----------|--------------|----------------|------|-------|-------|-------|--------|--------|
| | | | 1:20-1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1;1280 | 1;2560 |
| Cattle | 74 | 63 | 31 | 6 | 7 | 6 | 6 | 6 | 1 |
| Sheep | 28 | 12 | - | - | - | - | - | - | - |
| Goats | 19 | 11 | - | - | - | - | - | - | - |

Results of microscopic agglutination test live antigen on cattle serum samples at the Royal Tropical Institute, Amsterdam with different leptospira serogroups are listed in table (5). The results of the work conducted at the public health laboratory service U.K. were examined by microscopic agglutination test using formalized cultures of leptospira are summarized in table (6). Eighteen out of 75 cows serum samples were positive at dilution from 1:100 to 1:800. Twenty nine sheep serum samples and 19goats serum samples were negative to all of the serotypes used in the test.

Table (5) Results of microscopic agglutination Test -live antigen-on cattle serum samples at the Royal Tropical Institute Amsterdam with different Leptospira serogroups

| Ser. No, | Lep. serogroup. | Serum dilution | | | | | | Total |
|----------|-----------------|----------------|-------|-------|-------|--------|--------|-------|
| | | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | |
| 1 | Sejroe | 2 | 5 | 6 | 4 | 5 | 1 | 23 |
| 2 | Mini | 5 | 3 | 1 | 2 | 1 | - | 12 |
| 3 | Hebdomadis | - | 1 | 3 | 2 | 2 | - | 8 |
| 4 | Semaranga | 4 | 1 | - | - | - | - | 5 |
| 5 | Javanica | 1 | 2 | 1 | - | - | - | 4 |
| 6 | Grippotyphosa | 2 | - | - | 1 | - | - | 3 |
| 7 | Pomana | - | - | 1 | 1 | - | - | 2 |
| 8 | Tarrassovi | - | - | - | - | 1 | - | 1 |
| 9 | Autumnalis | - | - | 1 | - | - | - | 1 |

Table (6) Results of Microscopic Agglutination Test -Killed Antigen- on Cattle, Sheep, and Goats serum samples at Public Health Laboratory Service U.K

| Animal species | No. examined | No. positive | Serum Dilution | | | |
|----------------|--------------|--------------|----------------|-------|-------|-------|
| | | | 1:100 | 1:200 | 1:400 | 1:800 |
| Cattle | 75 | 18 | 6 | 8 | 3 | 1 |
| Sheep | 29 | - | - | - | - | - |
| Goats | 19 | - | - | - | - | - |

Table (7) Results of examination of serum samples with Macroscopic Plate Agglutination Test using pool leptospira antigen

| Leptospira antigens | Cattle | | Sheep | | Goats | |
|---------------------|-------------------|-------|-------------------|-------|-------------------|-------|
| | Total N0. exam./+ | +ve % | Total N0. exam./+ | +ve % | Total N0. exam./+ | +ve % |
| Pool 1 | 83/0 | 0 | 31/0 | 4.1 | 44/0 | 0 |
| Pool 2 | 260/27 | 10.4 | 171/4 | 2.3 | 134/5 | 3.7 |
| Pool 3 | 260/42 | 16.2 | 171/15 | 8.8 | 134/12 | 8.9 |
| Pool 4 | 260/18 | 6.9 | 171/7 | 4.1 | 134/5 | 3.7 |

*Pool 1 containing: *L.ballam*, *L. canicola*, *L.icterohaemorrhagiae*.

Pool 2 containing: *L.bataviae*, *L.gripotyphosa*, *L.pyrognos*.

Pool 3 containing: *L.autumnalis*, *L.pomona*, *L.wolffi*.

Pool 4 containing: *L.australis*, *L.hyos*, *L.georgia*, *LT 117*.

Discussion

The results of the present study have indicated the prevalence of leptospirosis in cow 5.703% sheep 24.6% and goats 22.4%. The prevalence of leptospirosis in cattle in and around Baghdad was fairly high, though the prevalence was low in sheep and goats in Baghdad. It is quite necessary and important to give more attention and proximity to animals and with an occupational risk of exposure (12). Tan in 1964 found that 29.6% of patients with pyrexia of unknown origin had antibodies indicative of leptospirosis. Results from table (7) indicate that pool 3 was the common antigens in cattle, sheep and goats. Next in order being pool 2 in cattle with 10.4 % and in sheep with 4.1 % positive reaction. *Leptospira Pomona* member of pool 3 has been reported to be common serotypes affecting cattle and sheep (14) (3) (7). *Leptospira grippotyphosa* member of pool 2 has also reported in cattle and goats (15) (16) (17).

Results of table (4) revealed titers from 1:80 to 1:2560 were found only in cattle, where as in sheep and goats titers were below 1:80. This indicated that there was a constant source of infection as a result of which titers were due to anamnesis response. The low titer may be due to the fact that the samples were collected from slaughter house where the age of such animals was less than one year, as such changes of being exposed to repeated infection were much less. The results of table (6) revealed that only cattle sera had given positive reaction when tested with killed antigens. The negative results in respect of sheep and goats serum samples may be due to very low titers which may not have been detected by this method.

However the macroscopic plate agglutination test has been recommended as a screening test of animals and human sera (18) (19) (9) (20) thought it is less sensitive than microscopic agglutination test. It detects any exposure during previous 1-2 years (21).

The present findings have opened a new avenue for further research in this field, since this is the pioneer study in Baghdad province about leptospirosis in domestic animals.

Macroscopic plate agglutination test has been found to be satisfactory, though not highly sensitive, as a screening test in preliminary serological survey to identify the prevalence of the disease. It is not possible to conduct microscopic agglutination test in ordinary laboratories due to obvious difficulties of maintaining live culture of several serotypes and risk of human of exposure. It is therefore necessary to depend on macroscopic plate agglutination test.

In a country like Iraq where dairying is a growing industry it is essential to establish the definite causes of abortion in cow. In every case of abortion there is a tendency to consider brucellosis as the most probable cause, since this study has proven that aborted cattle showed high antibody titers by microscopic agglutination test, it was highly suggestive that leptospirosis may be associated with abortion. It is recommended that similar investigation be extended to survey different parts of the country in order to get on overall picture of the leptospirosis in Iraq. Such survey may also be conducted on other species of domestic animals such as horses and dogs as they are considered to be potential source of infection to man and other animals. Finally it is emphasized that further work will be necessary to include the isolation and typing leptospiral organisms.

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Study of some immunization effects against attenuated *Pseudomonas aeruginosa* in local rabbits

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Abstract

The study was carried out on the 12 local rabbits, divided into four groups, the first one was injected by a stock solution of attenuated *Pseudomonas aeruginosa* ($0.1\text{ml } 26 \times 10^{-4}$), the second and third group injected by 1/2 and 1/4 dilution respectively, while the last fourth group injected by normal saline and considered as control group. Our results showed significant variations in hypersensitivity test of immunized group in comparison with the control group. The results of hepatomegaly and splenomegaly showed valid decrease in first dilution immunized group. Significant enlargement of lung and kidney were found in control group while minimum weight recorded second dilution immunized group. There was significant increase of IgG level of immunized group in compared with control group. The level of complement (C3 & C4) showed significant increase in C4 of immunized group in comparison with the control group.

دراسة بعض مظاهر التمنيع ضد الإصابة بـ *Pseudomonas aeruginosa* في الأرانب المحلية

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الخلاصة

أجريت هذه الدراسة على 12 أرنباً محلياً وزعت في أربع مجاميع حققت المجموعة الأولى بمحلول البكتيريا المضعفة وحققت المجاميع الثانية والثالثة بالتخفيف 2/1، 4/1 على التوالي في حين حققت المجموعة الأخيرة بالمحلول الفسلجي واعتبرت كمجموعة سيطرة. أظهرت النتائج فروقا معنوية في اختبار فرط الحساسية للمجاميع الممنعة بالمقارنة مع مجموعة السيطرة، كما أظهرت نتائج تضخم الكبد والطحال انخفاضاً واضحاً في مجموعة التخفيف الأولى، وارتفاعاً معنوياً في تضخم الرئة والكلى في مجموعة السيطرة وسجل أقل الأوزان في المجموعة الممنعة بالتخفيف الأول والثاني. كذلك أظهرت النتائج ارتفاعاً معنوياً في تركيز الأجسام المضادة نوع وارتفاعاً في مستوى تركيز 4 في مصل الأرانب الممنعة مقارنة مع مجموعة السيطرة.

Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic rod, belonging to the bacterial family *Pseudomonadaceae*. (1) The family includes *Xanthomonas*, which together with *Pseudomonas*, comprise the informal group of bacteria known as Pseudomonads (2), These bacteria are common inhabitants of soil and water, They occur regularly on the surfaces of plants and occasionally on the surfaces of animals. The pseudomonads are better known to microbiologists as pathogens of plants rather

than animals, Since *P. aeruginosa* can live in both inanimate and human environments, it has been characterized as a “ubiquitous” microorganism.(3). *Pseudomonas aeruginosa* is a major cause of nosocomal and community acquired chronic infection and has high level of innate antimicrobial resistance, This has led researchers to investigate vaccine and immunotherapeutic approaches to prevent and treat *P. aeruginosa* infection (4). *P. aeruginosa* groups tend to form biofilms, which are complex bacterial communities that adhere to a variety of surfaces, including metals, plastics, medical implant materials, and tissue, biofilms are characterized by “attached for survival” because once they are formed, they are very difficult to destroy. Depending on their locations, biofilms can either be beneficial and detrimental to the environment, for instance, the biofilms found on rocks and pebbles underwater of lakes and ponds are an important food source for many aquatic organisms; on the contrary, those that developed on the interiors of water pipes might cause clogging and corrosions (5,6). *P. aeruginosa* produces two extracellular protein toxins, Exoenzyme S and Exotoxin A in addition to Lipopolysaccharide of outer membrane, purified Exotoxin A is highly lethal for animals including primates(1). This exoproduct is responsible for direct tissue destruction in lung infection (7) It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections (8). *P. aeruginosa* produces several extracellular products that after colonization can cause extensive tissue damage, bloodstream invasion, and dissemination, In vivo studies have shown that mutants defective in the production of exotoxin A, exoenzyme S, elastase, or alkaline protease are essential for maximum virulence of *P. aeruginosa*; however, the relative contribution of a given factor may vary with the type of infection (9). The our research point toward studying possibility of vaccines productions to prevent infection by *P. aeruginosa*.

Materials and Methods

1. **Vaccine:** Attenuated live cells of *Pseudomonas aeruginosa* was prepared as described by (10) and used for experimental animal vaccination , bacterial count was done by method mentioned by Tomasiewicz (11).
2. **Rabbits:** apparently healthy, local rabbits were used. A total of 12 animals were divided to four groups, reared in separated cages and fed green food. Animals in first group were immunized with 0.1ml stock solution of attenuated bacteria 26×10^{-4} by intradermal injection. The second and third groups immunized with $\frac{1}{2}$ and $\frac{1}{4}$ dilution of stock solution respectively, While the last fourth group injected with 0.1ml of normal saline and was considered as control group.
3. **Hypersensitivity reaction:**
 - A. The procedure was made as described by Bacharach (12) et al. After first dose of the vaccine, hypersensitivity reaction was evaluated by measuring increase in the thickness of skin at the site of injection and length of redness area as well as increase in the temperature.
 - B. **Second dose:** After 14 days, a second dose was given to each animal in the same amount and concentration of first dose and the reaction of hyper sensitivity was read in the first 24hrs, 48hrs, 72hrs and 96hrs respectively.
 - C. **Challenge dose:** After 28 days challenge dose 0.06ml of stock solution was given intranasal to all animals in all groups.
- **Weighting of Liver, spleen and lung:** After 3days the liver, spleen and lung of each animal of all groups were weighed.

- **Differential white blood cells counts** : Differential WBCs count evaluated as described by Al Dragee et al (13).
- **Statistical Analysis:** The results were analyzed by using Complete Randomized Design for identifying of the effect of different treatment in different cases and using of least significant differences among median of treatments to identifying significant differences according to the Franey et al (14).

Results

The results of our study showed the effect of three different concentration of the thickness of the skin in comparison with control group. maximum significant thickness increase was present after 24hrs in all three groups as compared with the control. Additionally, significant increase of stock solution and first dilution as compared with the second concentration . Also there was significant increase in thickness after 24hrs and 48hrs as compared with 72hrs, 96hrs and the thickness before injection in stock solution group. Table (1) summarizes effects of time and concentration on thickness of skin in all tested groups.

Table (1) Mean of rabbits skin thickness evaluated by millimetres before and after intradermal injection by attenuated *Pseudomonas aeruginosa*

| | Before | After 24hrs | After 48hrs | After 72hrs | After 96hrs |
|--|--------------------|---------------------|---------------------|----------------------|---------------------|
| Stock | 1.33 0.33± b | 3.33 0.88± Aa | 3.16 0.72± Aa | 2.00 0.57± Ab | 2.00 0.57± Ab |
| 1st dilution 1/2 | 1.33 0.33± b | 3.33 0.88± Aa | 2.00 0.57± Bb | 1.66 0.33± ABb | 1.66 0.33± B |
| 2nd dilution 1/4 | 1.33 0.33± | 2.50 0.29± B | 1.33 0.33± C | 1.33 0.33± B | 1.33 0.33± B |
| Control | 1.33 0.33± a | 1.33 0.33± Ca | 1.33 0.33± Ca | 1.33 0.33± Ba | 1.33 0.33± Ba |

Different capital letters refer to significant variation between different groups.

Different small letters refer to significant variation between different periods.

The results showed significant presence of redness area after 24hrs of injection by all three different concentration in comparison with animals state before injection. The control group did not show any redness area after injection with normal saline. The static differences and mean of diameters were explained in Table(2).

Table (2) Diameters mean of redness area evaluated by centimeters before and after intradermal injection by attenuated *Pseudomonas aeruginosa*.

| | Before injection | After 24hrs | After 48hrs | After 72hrs | |
|--------------------------------|------------------|----------------------|----------------------|-------------|------------|
| Stock | Zero Ac | 15.00 1.52± Aa | 9.33 0.33± Ab | Zero Ac | Zero Ac |
| 1st dilution | Zero Ab | 7.33 3.69± Ba | 4.30 2.18± Ba | Zero Ab | Zero Ab |
| 2nd dilution | Zero Ab | 4.00 3.98± Ca | 3.33 3.31± Bab | Zero Ab | Zero Ab |
| Control | Zero Aa | Zero Da | Zero Ca | Zero Aa | Zero Aa |

Different capital letters refer to significant variation between different groups.

Different small letters refer to significant variation between different periods.

The immunized animals showed slight increase in temperature, while control group kept normal level of temperature. The difference among group did not reach the level of significance (Table 3).

Table (3) Temperature (C°) of rabbits before and after intradermal injection by attenuated *Pseudomonas aeruginosa*

| | Before injection | After 24hrs | After 48hrs | After 72hrs | After 96hrs |
|--------------------------------|------------------|----------------|----------------|----------------|----------------|
| Stock solution | 37.76 0.27± | 37.96 0.27± | 37.17 0.61± | 37.40 0.32± | 33.40 0.32± |
| 1st dilution | 37.80 0.15± | 37.87 0.29± | 37.44 0.29± | 37.30 0.27± | 37.3 0.27± |
| 2nd dilution | 37.60 0.21± | 38.2 0.1±2 | 38.16 0.38± | 38.1 0.38± | 38.1 0.38± |
| Control | 37.63 0.2±4 | 37.63 0.24± | 37.63 0.24± | 37.63 0.24± | 37.63 0.24± |

After 14 days, the animals retreated with same first dose, the results of thickness revealed significant increase skin thickness of stock solution group after 24hrs and 48hrs as compared to thickness of skin after 72hrs as well as in compared with control group which re-injected with same dose of normal saline. The differences among all groups illustrated in (Table 4).

Table (43) Means of rabbits skin thickness evaluated by millimetres before and after i/d 2nd injection by attenuated *Pseudomonas aeruginosa*

| | After 24hrs | After 48hrs | After 72hrs |
|--------------------------------|---------------------|----------------------|---------------------|
| Stock | 3.18 0.73± Aa | 2.63 0.41± aab | 2.01 0.47± Ab |
| 1st dilution | 2.17 0.38± B | 1.42 0.23± B | 1.32 0.19 B |
| 2nd dilution | 2.29 0.41± Ba | 1.78 0.22± Bab | 1.28 0.06± Bb |
| Control | 1.33 0.33± C | 1.33 0.33± B | 1.33 0.33± B |

Different capital letters refer to significant variation between different groups.

Different small letters refer to significant variation between different periods.

The mean of diameters of redness area revealed significant increase of stock solution group in comparison to first dilution and second dilution as well as control group. Both first and second dilution showed significant increase in redness area as compared with control group. Also significant increase in diameter of stock solution group after 24hrs to state of animals after 72hrs. The correlation among groups and significance variation levels illustrated in Table (5).

Table (5) Diameter means of redness area evaluated by centimeters before and after intradermal injection by attenuated *Pseudomonas aeruginosa*

| | After 24hrs | After 48hrs | After 72hrs |
|--------------------------------|----------------------|----------------------|-------------|
| Stock | 11.49 0.56± Aa | 8.10 0.80± Aab | Zero Ab |
| 1st dilution | 5.79 0.15± B | 5.35 0.31± AB | Zero |
| 2nd dilution | 5.44 2.32± B | 1.67 1.66± Bc | Zero |
| Control | Zero C | Zero C | Zero |

Different capital letters refer to significant variation between different groups.

Different small letters refer to significant variation between different periods.

The animals in all groups injected intranasal by challenge dose of *Pseudomonas aeruginosa*. After 3 days weighing of immune organs showed that enlargement of stock solution injected group and control group as compared with 2nd group and 1st dilution group that give minimum liver weight. The spleen did not show high differences (Table 6, Fig.1 and Fig. 2) Other important organ lung and kidney were weighed the result showed significant enlargement of lung in case of stock solution group, first dilution and control group as compared to second dilution group (minimum level of lung weight). The mean of kidney weight results showed enlargement of kidney in case of stock solution group, first dilution and control group as compared with second dilution (minimum weight of kidney) as explained in table (Table 6).



Fig. (1) The difference between liver size of immunized group (left side) and control group (right side)

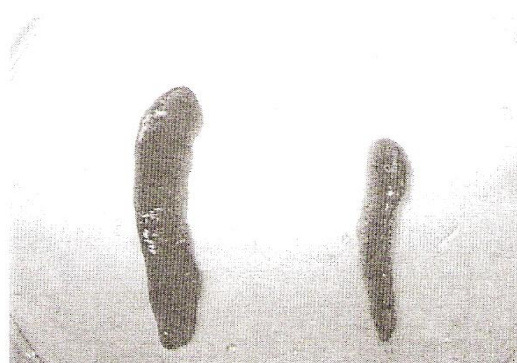


Fig. (2) The difference between spleen size of immunized group (right side) and control group (left side)

Table (6) The percent ratio of weight of immune organ to weight of animal show the difference between subjected animals and control group

| | Stock solution | 1 st dilution | 2 nd dilution | Control |
|---------------|----------------|--------------------------|--------------------------|---------|
| Liver | 3.56 | 2.35 | 2.68 | 3.11 |
| | 0.020 | 0.840 | 0.070 | 0.780 |
| Spleen | 0.043 | 0.036 | 0.043 | 0.043 |
| | 0.003 | 0.003 | 0.007 | 0.009 |
| Lung | 0.553 | 0.646 | 0.386 | 0.766 |
| | 0.098 | 0.018 | 0.072 | 0.049 |
| | AB | A | B | A |
| Kidney | 0.996 | 1.106 | 0.743 | 0.966 |
| | 0.022 | 0.029 | 0.009 | 0.083 |
| | B | A | C | B |

Different capital letters refer to significant variation between different groups.

Table (7) The mean of total and differential WBC count of rabbits in different groups

| | Stock | 1 st dilution | 2 nd dilution | Control |
|--|-------|--------------------------|--------------------------|---------|
| WBC (10³/mm³) | 49.33 | 48.00 | 54.67 | 54.67 |
| | 0.66 | 1.15 | 5.67 | 5.67 |
| N(%) | 60.33 | 52.30 | 53.66 | 59.66 |
| | 3.65 | 2.32 | 3.17 | 5.87 |
| E(%) | 4.33 | 4.66 | 4.00 | 3.33 |
| | 0.33 | 2.59 | Zero | 1.67 |
| M(%) | 8.33 | 16.66 | 18.33 | 17.66 |
| | 4.31 | 2.02 | 2.01 | 8.37 |
| L(%) | 24.00 | 26.00 | 22.33 | 19.00 |
| | Zero | 1.99 | 0.33 | 3.77 |
| B(%) | Zero | Zero | Zero | Zero |

The total IgA, IgG, C3 and C4 were investigated the results showed significant increase in IgG level and C4 as compared to control group (Table 8) and (Table 9).

Table (8) The differences between concentration of immunoglobulins (IgA and IgG) in both treated and control group

| | IgA | IgG |
|------------------------|----------|----------|
| Treatment group | 185.66 | 765.33* |
| | 12.01± | 51.31± |
| Control group | =125.66X | =504.33X |
| | 12.06± | 73.79± |

*significant increase

Table (9) The differences between concentration of complement component (C3, C4) in both treated and control group

| | C3 | C4 |
|------------------------|--------|-------|
| Treatment group | 144.66 | 42.5* |
| | 10.40 | 5.5 |
| Control group | 120.33 | 11.50 |
| | 14.57 | 0.5 |

*significant increase

Discussions

All animal groups injected by attenuated *Pseudomonas aeruginosa* assessed by delayed hypersensitivity test exhibited specific immunological response. Our results showed significant increase in thickness of skin as well as sensitive area that appear red, the highest mean was present in stock solution after 24 and 48hrs that reach to

(3.33mm While redness area 15cm), (3.16mm While redness area 9.33mm) respectively, also there was slight significant increase in temperature. The results are in agreement with those obtained by (15,16) who used antigen of *Entameba hisyolotyica* to induce skin hypersensitivity reaction. The second dose after 14 days demonstrated significant increase in thickness of skin and highest mean was present in first group which injected by stock solution (3.18mm) after 24hrs. The same group revealed highest mean in redness (11.49mm) after 24hrs. The thickness of skin may be due to aggregation of T-cells and releasing of cytokines that attract cells & other inflammatory cells at site of reaction, while the redness explained by increase blood vessels permeability (17).

Our finding showed increase in liver weight of control & stock solution groups this finding is in agreement with that obtained by (18), this result may refer to effectiveness of 1st dilution to increase resistance against infection.

Our results showed that the subjected animals to doses of bacteria will stimulate immune system in response to largest dose of bacteria even at specific site of infection, this result is in agreement with (18,19) results who found that serum obtained from vaccinated rabbits was able to confer temporary protection to mice against challenge with homologous or hetrologous strain of *Pseudomonas*.

The immunological parameters showed significant increase in level of IgG. The result is in agreement with (21) and in agreement with (22) who reported significant increase of 24.6% of IgG antibodies against *P.aeruginosa* in patients with cystic fibrosis (CF) in human.

Our results show non significant increase in level of secretory IgA, however, Herbert et al (19) found increase level of IgG& IgA this may be down to Herbert study rely on secretion, while our study deal with serum immunoglobulins.

C3 has no significant increase this result is in agreement with those obtained by other investigators (23). *P.aeruginosa* evade human complement attack by binding the human plasma regulator factor H and factor H-related protein-1(FHR-) to its surface. Similarly factor H bound to intact *P. Aeruginosa* showed complement regulatory activity and mediated C3b degeradation.

The significant increase in the level of C4 recorded in this study was similar to those ontained by Shaker (14) who eported significant increase of C4 in case of immunization against *Entamoeba histolotyica* in New Zealand rabbits and this may be due to presence of antigen- antibody complex that lead to complement activation.

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The effect of local application black seed (*Nigella sativa*) oil on wound healing in rabbits

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Abstract

The objective of this study is to determine the effectiveness of topical application of black seed oil on the wound healing in rabbits. Twenty adult healthy local breed rabbits of both sexes weighing between 1250-1600 gr were used. Animals were divided into two equal groups, under surgical aseptic technique, two surgical skin incisions at length of 3 cm. in the back region were done one each sides of the vertebrae, then they were closed with simple interrupted pattern by silk (2\0). Control group didn't receive any treatment, while in treated group, the wounds were covered with black seed oil twice daily for 14 days. The clinical and histopathological evaluation revealed that black seed oil promote the wound healing by early formation of cellular fibrous connective tissue and granulation tissue and early maturation of fibrous connective tissue, which characterized by regular and less cellular covered by complete layer of epidermis, when compared with control group. In conclusion of this study indicated that the black seed oil was enhanced wound healing, and that may be due to its therapeutic and nutritional activities.

تأثير زيت الحبة السوداء المستخدم موضعياً على التئام الجروح في الأرانب

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فرع الجراحة والتوليد - كلية الطب البيطري / جامعة بغداد

الخلاصة

استهدف البحث دراسة تأثير الاستعمال الموضعي لزيت الحبة السوداء على التئام الجروح في الأرانب، استعمل في التجربة عشرون أرنباً بالغاً من كلا الجنسين تراوحت أوزانها ما بين 1250-1600غم. قسمت الحيوانات إلى مجموعتين متساويتين وبعد تحضيرها جراحياً وتخديرها عمل شقين جراحيين في الجلد بطول 3 سنتمتر في منطقة الظهر على جانبي الخط الظهري، خيط الجرح بطريقة المتقطع البسيط باستعمال الحرير قياس 0\2. مجموعة السيطرة تركت للشفاء الطبيعي بدون أي معالجة، في حين استعمل في مجموعة المعالجة زيت الحبة السوداء موضعياً مرتان يومياً لمدة 14 يوم. أظهرت نتائج الفحص العياني والنسجي المرضي ان زيت الحبة السوداء قد ساعد في شفاء الجروح عن طريق التكوين المبكر للنسيج الحبيبي وكذلك نضوج النسيج الضام والذي تميز بانتظام أليافه واكتمال طبقة البشرة التي تغطيه. نستنتج من هذه الدراسة بأن زيت الحبة السوداء يساعد في تسريع التئام الجروح.

Introduction

Nigella sativa (*N.sativa*) seeds, called as Black seeds in English language, *Al-habba Al-sauda* or *Habbatul -barakah* in Arabic and *Kalvanji* in some local languages in the Indian subcontinent, is well known in the Middle East, Middle Asia and Far East; as a natural remedy for many ailments and flavoring agent in bread and pies. There is common Islamic belief that Black seed is a remedy of all ailments but that it cannot prevent aging or death (1). Many studies have been carried out in the last few decades on the pharmacological effect of *N. sativa* and its active principles these studies and the advancements in the methods of analysis have led to discovery of many active principles like; proteins, alkaloids, saponin (melanin), fixed and essential oil (2 and 3). Crude fiber, calcium, iron, sodium, and potassium are also present. Nutritional composition of the seeds has been determined as 21% protein, 35% carbohydrate, and 36% fat (4). It's appear that the compounds in the oil act synergistically so that it is important to use the whole oil or crude extract of the seeds in pharmacological studies (5). The plant extract and its essential oil showed a broad range of pharmacological effects such as: anti-diabetic (6) spasmolytic and bronchodilator (7), antioxidant (4) hepato-protective (8), analgesic and anti-inflammatory antipyretic (9), antitumor (10) anti-bacterial, anti-fungal (11), antiviral (12), immune stimulation (13).

The injury of any type triggers is an organized and complex serial cellular and biochemical events that result in a healed wound. These processes can lead to pathological conditions if healing is excessive or deficient, wound healing failures can pose a significant clinical problem with a large impact on morbidity, mortality and medical costs (14). Thus the healing can be enhanced by controlling the local and systemic factors that influenced it like :- The quality of the vascular supply to the area, the presence of a deleterious infection, mechanical stress on the wound, abrasive or inflammatory suture material, radiation injury, hypoproteinaemia and hypovolaemia, edema, malnutrition and vitamin deficiency, administration of corticosteroids, diabetes mellitus, the administration of cytotoxic drugs, jaundice, uremia and advanced age (15).

Because of the characteristic properties of black seed it has been used in the treatment of many types of wound and trauma (16, 17). In this study black seed oil was topically applied on induced surgical wound in rabbit to evaluate its effect on wound healing clinically and histo-pathologically.

Materials and Methods

Twenty adult healthy local breed rabbits of both sexes weighing between 1250-1600 gr were used. The animals were divided randomly into two groups: control group (n=10) and treated group (n=10). The back region was prepared surgically, then the animals were anesthetized by intra-muscular injection of mixed (40 mg /kg Ketamine HCl, 10 mg Xylazine and 4mg/kg diazepam) (18). Two paravertebral straight incisions of 3 cm length were made through the entire thickness of the skin at a distance of about 4 cm. The skin was closed by simple interrupted sutures using silk (size 2/0). The animals of treated group in addition to the same procedures as in group one they were treated by topical application of black seed oil (Emmad factory for oil production, Mosul, Iraq permit no. 70490) on the wound, twice daily for 14 days. In both groups specimens for histopathological examination were collected at the day 3rd, 5th, 7th and 14th post operation, the specimens were fixed in buffered formalin 10 % and routine preparation of the section, then stained with Hematoxylin and Eosin (19).

Results

Histopathological examination of the control group, revealed that at 3 days post operative the section showed presence of necrosis and inflammatory cells infiltration in the incision site consist mainly from neutrophils (Fig. 1), also in other section a network of fibrin were seen in the dermis and inflammatory cells which was mainly neutrophils and fibroblasts began to replace the fibrin network (Fig.2). At 5 days post-operative the site of incision filled with immature granulation tissue consist from angioblast and fibroblast in the dermis which covered by thick epidermis layer (Fig. 3). The section of 7 days post-operative there is fibrous connective tissue proliferation which characterized by more collagen deposition, moderate irregular direction and sever thickness of epidermis, formed papillae extend to the dermis (Fig.4).While at 14 days post-operative the microscopic lesion showed proliferation of immature fibrous connective tissue which infiltrated by mononuclear cells and proliferation of stratified squamous cell epithelium which extended at bridge into dermis with more basophilic basal cells (Fig.5).

In the treated group, the histopathological examination was revealed that at 3 days postoperative the lesion was characterized by inflammatory cells infiltration mainly neutrophils and in the same section area, there was beginning of the proliferation of fibroblast (Fig. 6), in another section the lesion showed immature cellular fibrous connective tissue, with capillary blood vessels (granulation tissue) which present in the incision site (Fig.7). While at the 5 days, more cellular fibrous connective tissue were present in the sutured area with moderate thickness dermis layer were seen (Fig. 8). At the 7 days postoperative more regular dense collagen fibers with mononuclear cell infiltration were seen in the section area covered by complete epidermal layer (Fig 9).At 14 days ,more mature fibrous connective tissue was present in the section area characterized by regular and less cellular covered by complete layer of epidermis (Fig.10).

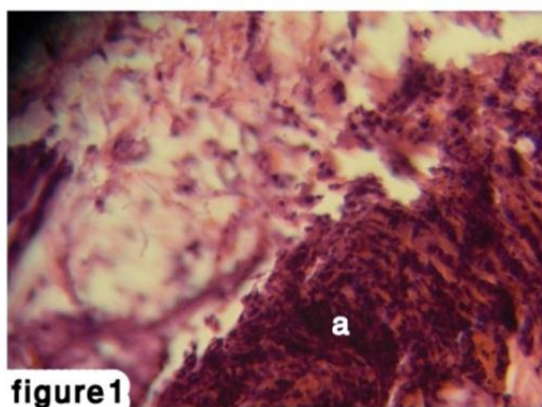


figure1
Fig .(1) Histological section of skin of control group at 3 days post operative showed necrosis and inflammatory cell infiltration in the incision site consist mainly from neutrophil (a) H & E X 40.

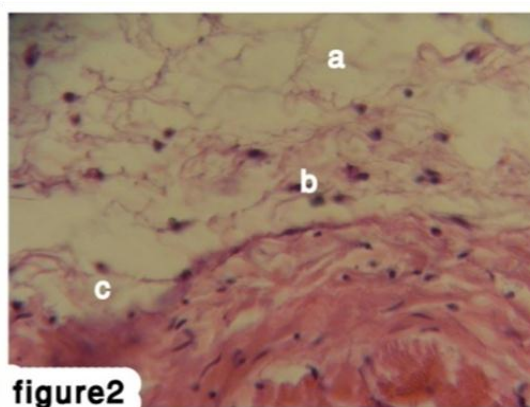


figure2
Fig. (2) At 3 days postoperative, network of fibrin (a) are seen in the dermis, and inflammatory cell mainly neutrophil (b), and fibroblast began to replace the fibrin network (c) H & E X 40.

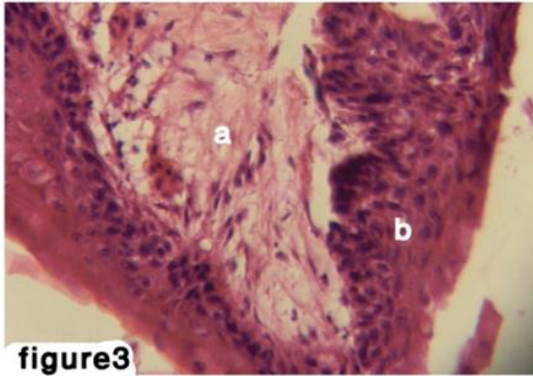


figure3

Fig (3) At 5 days post operative, the site of the incision filled with immature granulation tissue (a), consist from angioblast and fibroblast in the dermis which cover by thick epidermis layer (b) H & E X 40.

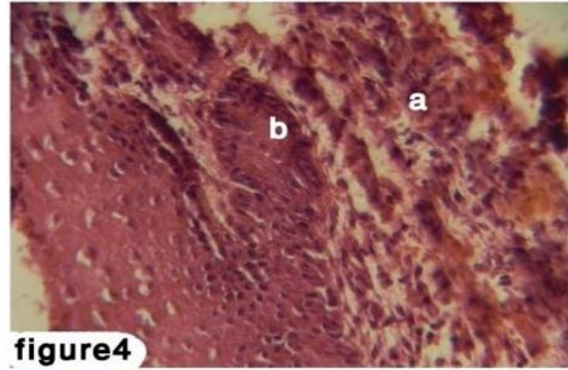


figure4

Fig. (4) At 7 days postoperative there is fibrous connective tissue proliferation characterized by more collagen deposition, moderate irregular direction (a) and sever thickness of epidermis which form papillae (b) extend to the dermis H & E X 40.

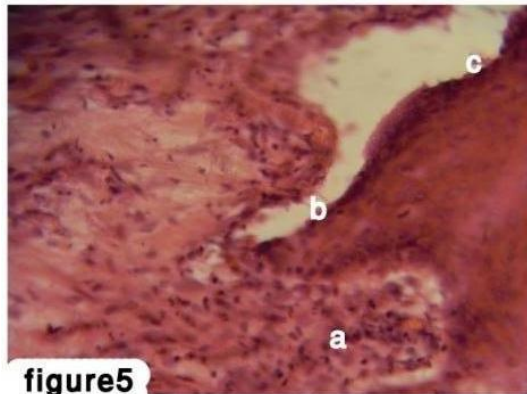


figure5

Fig. (5) At 14 days postoperative, the proliferation of immature fibrous connective tissue which infiltrated by mononuclear cell (a) and proliferation of stratified squamous cell epithelium which extended at bridge into dermis(b) with more basophilic basal cell (c) H & E X 40.

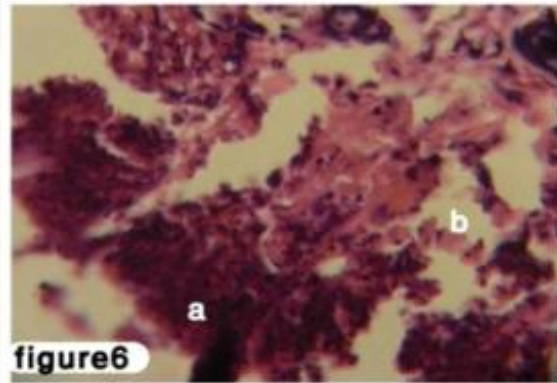


figure6

Fig. (6) At 3 days postoperative of treated group, inflammatory cell infiltration mainly neutrophils (a) with beginning proliferation of fibroblast (b) H & E X 10.

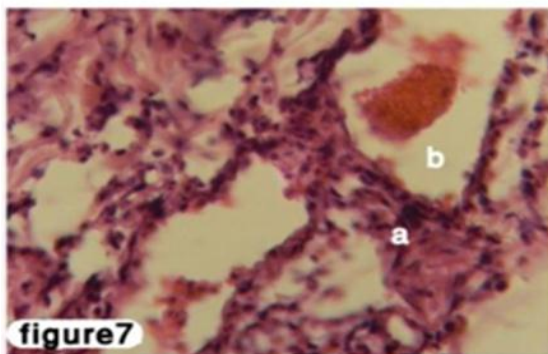


figure7

Fig. (7) At 3 days postoperative of group two immature cellular fibrous connective tissue (a) with capillary blood vessel (b) (granulation tissue) is present in the section area H & E X 40.

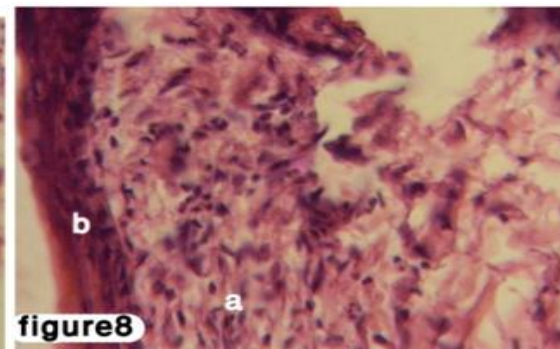


figure8

Fig. (8) At 5 days postoperative: more cellular fibrous connective tissue are present in the sutured area (a) with moderate thickness dermis layer are present (b) H & E X 40.

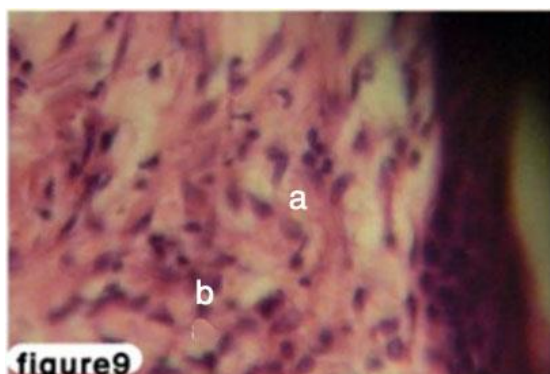


Fig. (9) At 7days postoperative: more regular dens collagen fibers (a) with mononuclear cell (b) infiltration is seen in the section area covered by complete epidermal layer H & E X 40.

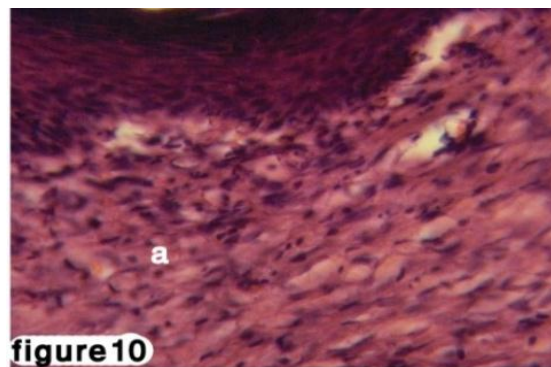


Fig. (10) At 14 days postoperative more mature fibrous connective tissue present in the section area which are regular and less cellular (a) covered by complete layer of epidermis H & E X 40.

Discussion

The goal of wound reconstruction is to return the individual to the best possible function as quickly as possible and with the best cosmetic results (20), innumerable substances and methods have been used, either locally or systemically to achieve this goal. Some examples are: prophylactic administration of antibiotics, medicinal plants (17 and 21), honey (22), lasers (23). Most of these therapies were found ideal and had wide success in promotion of wound healing.

Wound healing generally requires support at three levels: first, improving general resistance and support, second, stimulating the repair and regenerative mechanisms, third, therapeutic and nutritional activities (24). Multitude of these requirements were well provided by *Nigella sativa*.

Histopathological examination of investigate this study that the proliferation phase was began at 3rd day of operation in the treated group, which characterized by granulation tissue formation, while this stage didn't start till 5th day in control group, this confirm that *N sativa* enhance production of human interleukin and alerts macrophages (13).

In otherwise macrophages are able to phagocytes bacteria and provide a second line of defense, they also secrete variety of chemotactic and growth factors such as fibroblasts growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), and interleukin -1 (IL-1) which appear to direct the proliferative phase (25). Collagen synthesis is stimulating by various growth factors (26), growth hormone is also known to promote the proliferation fibroblasts (27) and fibroblast proliferation form the granulation tissue, so this accelerates two phases of healing epithelization and collagenization; however it retards granulation and scar formation, beside that the *N sativa* oil contains fatty acids which build collagen (4) that's promote wound healing and maintain the skin elasticity.

This appeared histopathologically by epithelized the treated wound faster at 7th day post operative and less thickness dermis layer when compared with control group which was characterized by sever thickness of epidermis.

The black seed oil also act as occlusive dressing with good edge seals and can provide a barrier to migration of micro organisms into the wound, whereas bacteria have been shown to pass through 64 layers of moist gauze (28) and also keep the site moist and give a soft texture to the skin during the healing process, that's described improved wound healing under moist conditions (25). The moisture and nutritional activities of

black seed oil enhance debridement, neutrophils cell life and proteolytic enzymes action which lead to painless debridement (29) further these fibrin degeneration products are factors which stimulating macrophages to release growth factors into wound bed. Finally, when antioxidants can interfere the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers ,oxidative stress also plays an important role in impaired wound healing. Botanical with anti-oxidant or free radical scavenging activity thus can play a significant role in healing of wound (24). It can be suggested that the healing activity of black seed after local and even systemic administration may at least be in part due to its potent antioxidant activity (4). In addition to all above the antimicrobial, antifungal, antiviral activities of *N sativa* oil may lead to a clean wound healing without secondary infection.

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Effect of feeding Sodium Hydroxide Treated Rice Hulls and Date Stones on the Reproductive Performances of Awassi Ewes

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Abstract

Fifty seven Awassi ewes, aged 2 – 3 years, were used in a completely randomized design and allocated to three different diets, ten weeks before mating. Synchronization was commenced mating, using progesterone impregnated intravaginal sponge. Diets 1 was concentrate feed only. Diets 2 included 60% concentrate plus 40% alkali treated rice hulls (50 NaOH in 1L water/kg DM) and diets 3 was composed of 30% concentrate plus 30% ground date stone plus 40% treated rice hulls. The experimental diets were fed four weeks before and four weeks after mating and also six weeks before lambing, at a level of 150% of the maintenance requirements. Between flushing and steaming up, ewes were fed alfalfa and green barley crop at a maintenance level. After lambing ewes were fed green alfalfa ad. Lib. And 500 g/day per ewe concentrate mixture for two weeks, followed by green alfalfa only. For six weeks daily feed intake and weekly live body weights were recorded. A balance trial was carried out using three Awassi rams in a 3x3 Latin square design.

The type of the diets did not have a significant effect on ewes lambing percentage nor on lambs birth weight or growth rate, until eight weeks after lambing. However, date stone may have affected oestrus activity. The results suggest that using treated rice hulls and ground date stone up to 70% of concentrate diet was adequate to meet Awassi ewes requirement during mating and late pregnancy.

تأثير استخدام السبوس المعامل بهيدروكسيد الصوديوم وجريش نوى التمر على الاداء التناسلي
للنعا ج العواسية

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الخلاصة

شملت الدراسة سبعة وخمسون نعجة بعمر 2 – 3 سنة وزعت عشوائيا على ثلاثة انواع من العلائق قبل عشرة اسابيع من التسفيد. عوملت النعا ج بالأسفنجيات المهبلية المشبعة بهرمون البروجستيرون لتوحيد الشبق. كانت العليقة الاولى علف مركز فقط وشملت العليقة الثانية 60% علف مركز مع 40% سبوس معامل بهيدروكسيد الصوديوم واحتوت العليقة الثالثة على 30% علف مركز مع 30% جريش نوى التمر و 40% سبوس معامل. غذيت علائق التجربة اربعة اسابيع قبل وبعد التسفيد وكذلك ستة اسابيع قبل الولادة وبمستوى 150% من احتياجات الادامة.

غذيت النعاج خلال الفترة بين التسفيد و الولادة بالجث وحشيش الشعير بمستوى الادامة. بعد الولادة غذيت لمدة اسبوعين بصورة حرة على الجث مع 500غرام/ يوم للنعجة علف مركز. تبعها علف اخضر فقط لمدة ستة اسابيع حسب كمية العلف المستهلك يوميا كما تم تسجيل وزن الجسم اسبوعيا. تم اجراء تجربة هضم باستخدام ثلاثة كباش وباستعمال تصميم 3 x 3 المربع اللاتيني.

لم يظهر أي تأثير معنوي للعلائق على نسبة الولادات او على الوزن عند الولادة وكذلك معدل النمو للحملات ولغاية ثمانية اسابيع من العمر ولكن اشر اختلاف العلائق على نسبة ظهور الشبق في النعاج. نتائج التجربة تشير الى امكانية سد احتياجات النعاج خلال فترات التسفيد وقبل الولادة بستة اسابيع باستخدام السبوس المعامل بهيدروكسيد الصوديوم وجريش نوى التمر ولحد 70% من العليقة المركزة.

Introduction

The effect of feeding Awassi ewes high plane of nutrition during mating and late pregnancy on their reproductive performance have been demonstrated by many workers (1, 2, 3). However few attempts were made to examine the effect of using agriculture by-products (mainly cereals straw) on ewes performance (4, 5). Most of the work concerning the use of agriculture by-products was conducted with fattening lambs (6).

Mohammed (6) found that NaOH treated rice hulls can be utilize successfully in fattening Awassi lambs, as reasonable fattening performance were achieved. Though, this experiment was designed to study and compare the performances of Awassi ewes fed complete diets comprising alkali treated rice hulls with or without date stones.

Materials and Methods

Fifty seven Awassi ewes (mean live weight and standard error 37.65 ± 0.55), aged 2 – 3 years were used in a completely randomized design. Ewes were penned indoors into three groups, ten weeks before mating and were mated in mid January after estrus synchronization, using progesterone impregnated intravaginal sponge*. Six Awassi rams wearing (sire sine) harnesses were introduced and mating date was recorded daily.

Ewes were allocated randomly to three different diets. Diet 1 (G1) was concentrate feed only. Diet 2 (G2) included 60% concentrate feed plus 40% alkali treated rice hulls (50 NaOH in 1L water/kg DM) and diet 3(G3) was composed of 30% concentrate plus 30% ground date stone plus 40% treated rice hulls. Table 1 shows the components of the three diets fed. The chemical analyses of the three experimental diets are shown in table 2. Alkali treated used was described by Mohammed (6). The experimental diets were fed four weeks before and four weeks after mating (flushing period) and also during the last 6 weeks of pregnancy (steaming up) at a level of 150% of the ewes maintenance requirement (7). During the period between flushing and steaming up, the ewes were fed alfalfa and green barley crop at a maintenance level. After parturition ewes were fed green alfalfa ad. Lib. And 500 g/day per ewe concentrate mixture for 2 weeks followed by green alfalfa only for six weeks.

Ewes feed intake was recorded daily and live body weights were recorded weekly and their weights were also recorded 24 hours after lambing. Lambs were weighted 2 hours after lambing and then weekly until 8 weeks of age.

*= Vermix , Up john Ltd.

Table (1) components of the experimental diets (%)

| Ingredient | Diet 1 | Diet 2 | Diet 3 |
|----------------------|---------------|---------------|---------------|
| Concentrate mixture* | 100 | 58.00 | 29.41 |
| Rice hulls | -- | 40.00 | 39.52 |
| Date stone | -- | -- | 29.41 |
| NaCl | -- | 0.40 | 0.40 |
| CaCO ₃ | -- | 0.80 | 0.77 |
| Urea | -- | 0.80 | 0.49 |

* = concentrate mixture (wheat bran 45% + barley 37% + soybean 12% +additives 6%)

A balance trial was carried out using three awassi rams in a 3x3 Latin square deign to determine digestion coefficients of dry matter, crude protein, crude fiber and energy. Total faeces voided were collected every day before feeding for seven days and ten percent fecal samples were bulked for each animal during the collection period and preserved at -10°C until were chemically analyzed. A 10-days preliminary period commenced each collection period (8).

Analysis of variance was applied to study the effect of type of diet on the ewe's performances. L.S.D. was used to compare between any two treatments. The data of live barren ewes were excluded from the late pregnancy results.

Results and Discussion

Lambing percentage was used as a preferred measure for describing the general fertility level in flock (9). Table 3 showed that no significant effect of type of diet on ewes lambing percentages (lambing percentage was 94.7, 89.4, and 89.4 for G1, G2 and G3 respectively). The results showed no incidence of twinning in all three groups. However, up to 20% twinning had been reported by younis (1), working on Awassi ewes. Whereas muhammed (3) found no significant effect of flushing on lambing percentage of Awassi ewes. Al-saigh et al. (2) found no significant effect of type of roughages (green, hay and silage) on the live weight changes of Awassi pregnant ewes.

Date stone had been used in the feeding of Awassi sheep (6, 10, 11 and 12). Hamra(12) showed that diet contained 50% date stone fed to breeding ewes effect their fertility. The results of the present experiment indicated that diet contained 30% date stone did not affect ewes fertility. However, date stone may have some effects on oestrus activity (Table 3). It was found that 40% or the ewes in group 3, who fed date stone, were returned to cycle. Hamra (12) suggested that date stone may have contained compound with estrogenic action.

The flushed ewes (Table 4) were increased in weights during the experiment period. No significant difference was observed between groups. The results in table 4 indicates that ewes fed 40% treated rice hulls and date stone can achieve similar performances to ewes fed concentrate diet only.

Ewes mean live weight and live weight changes during late pregnancy are presented in table5. All ewes of the three groups have gained weight during 6 weeks before lambing, no significant differences were observed between groups. However, live weight changes from 6 weeks before lambing to 24 hours post-lambing showed that G2 and G3 have lost weights, whereas G1 gained weight (Table 5). Jassim (14) found no significant effect of types of roughages (green, hay, and silage) on the live weight changes of Awassi pregnant ewes.

Type of diet had no significant effect on lambs birth weigh, growth rate until 8 weeks post lambing (Table 5). Lambs birth weights were 3.90, 4.25 and 4.35 kg for G1, G2 and G3 respectively. However, lambs birth weight for G2 and G3 had slightly heavier birth weights than G1.

The substitution of concentrate diet with agriculture by products (alkali treated rice hulls and ground date stone) up to 70% have reduced the digestion coefficients of dry matter, crude protein, crude fiber and energy significantly ($P<0.01$), table 6. This was probably due to the higher lignin, cellulose and ash content in diets 2 and 3 in comparison with diet 1 (Table 2).

It has been observed that pregnant ewes grazing estrogenic pasture affect their lambing performances (15). The main affect was abortion, uterine prolaps. However the present work did not show any effect on ewes fed 30% ground date stone. The results suggest that using treated rice hulls and ground date stone up to 70% of the concentrate diet was adequate to meet Awassi ewes requirement during late pregnancy.

Table (2) Composition of the three diets fed to experimental ewes

| | Diet 1 | Diet 2 | Diet 3 |
|----------------------------|--------|--------|--------|
| Dry Mater (g/kg) | 941.0 | 898.0 | 947.0 |
| Crude protein (g/kg Dm) | 140.0 | 138.0 | 143.0 |
| Crude fiber (g/kg Dm) | 93.8 | 158.2 | 315.4 |
| Ether extractive (g/kg Dm) | 21.8 | 5.1 | 8.8 |
| Ash (g/kg Dm) | 10.2 | 23.7 | 70.3 |
| NDF (g/kg Dm) | 768.6 | 540.9 | 301.9 |
| ADF (g/kg Dm) | 231.4 | 559.1 | 698.1 |
| Cellulose (g/kg Dm) | 72.7 | 128.4 | 250.4 |
| Hemi cellulose (g/kg Dm) | 128.2 | 291.1 | 253.3 |
| Lignin (g/kg Dm) | 20.3 | 104.2 | 121.3 |
| †Gross energy (MJ/kg Dm) | 18.84 | 18.31 | 17.80 |
| *ME (MJ/kg Dm) | 12.71 | 10.23 | 9.80 |

• = $0.81 \times \text{DE (8)}$

† = Gross energy was determined using a bomb calorimeter.

Table (3) Effect of feeding experimental diets on reproductive performance of Awassi ewes

| | G1 | G2 | G3 | Total mean |
|---|------|------|------|------------|
| No. of ewes | 19 | 19 | 19 | 57 |
| No. of ewes marked by rams | 18 | 16 | 16 | 50 |
| No. of ewes marked in 1 st oestrus cycle | 14 | 15 | 8 | 37 |
| No. of ewes marked in 2 nd oestrus cycle | 4 | 1 | 8 | 13 |
| No. of lambs born alive | 18 | 17 | 17 | 52 |
| No. of ewes lambbed | 18 | 17 | 17 | 52 |
| Barrenness | 1 | 3 | 3 | 7 |
| Lambing percentage (%) * | 94.7 | 89.4 | 89.4 | 91.1 |

* = No. of lambs born alive/No. of ewes joined with rams.

Table (4) Ewes feed intake and live-weight changes during flushing period

| | G1 | G2 | G3 | SIG | S.E.D |
|--|-------|-------|-------|-----|-------|
| No. of ewes | 19 | 19 | 19 | | |
| Ewes initial weight (kg) | 36.25 | 37.61 | 38.34 | | |
| Ewes weight at mating (kg) | 38.36 | 39.42 | 41.76 | NS | 1.5 |
| Ewes weight 3 weeks after mating (kg) | 41.4 | 42.13 | 44.18 | NS | 1.58 |
| Gain from start to 3 weeks after mating (kg) | 5.25 | 4.52 | 5.84 | NS | 0.69 |
| Dry matter intake (g)* | 1056 | 1260 | 1451 | | |

* = Ewes fed on a group basis; therefore no statistical analysis was carried out.

Table (5) Effect of feeding experimental diets on ewes performances (late pregnancy)

| | G1 | G2 | G3 | SIG |
|---|------------|------------|------------|-----|
| No. of ewes | 16 | 16 | 16 | |
| Ewes live-weight at start (kg) | 43.6 ± 1.3 | 44.1 ± 1.1 | 46.1 ± 1.2 | NS |
| Per-lambing ewes weight (kg) | 51.0 ± 1.6 | 48.5 ± 1.2 | 51.8 ± 1.5 | NS |
| Post lambing ewes weight (kg) | 45.1 ± 1.5 | 43.7 ± 1.1 | 45.0 ± 1.3 | NS |
| Live weight changes start-post lambing (kg) | 1.5 ± 0.5 | -0.3 ± 0.5 | -0.1 ± 0.7 | NS |
| Lambs birth weight (kg) | 3.9 ± 0.1 | 4.2 ± 0.1 | 4.3 ± 0.2 | NS |
| Lambs weight 8 weeks post-lambing (kg) | 12.4 ± 0.6 | 12.5 ± 0.5 | 12.6 ± 0.5 | NS |
| Lambs mortality (no) | - | 2 | 2 | |

Table (6) Digestion coefficients of DM, CP, CF and energy of diets 1, 2 and 3

| | G1 | G2 | G3 | S.E.D | SIG |
|---------------|-----------|-----------|-----------|--------------|------------|
| Dry matter | 0.79 | 0.66 | 0.66 | 0.070 | ** |
| Crude protein | 0.88 | 0.78 | 0.79 | 0.020 | ** |
| Crude fiber | 0.59 | 0.33 | 0.67 | 0.055 | ** |
| Energy | 0.81 | 0.69 | 0.69 | 0.029 | ** |

** Significant at the level ($P < 0.01$)

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Study the Inhibitory Effect of Green Tea Extracts on Growth of Some Dermatophytes

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Abstract

The antifungal profiles of green tea (*Camellia sinensis*) were examined against clinical isolates of Dermatophytes (*Trichophyton mentagrophytes*, *T. verrucosum* and *T. rubrum*) and some pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans*). Maceration method was used for the extraction of active substances from the green tea with cold and hot ethanolic and water extraction solvents. Agar dilution method was used in the antifungal susceptibility studies. This study revealed that the cold ethanolic extract was the most effective one, followed by the hot ethanolic extract while the aqueous extract was the least effective against all the tested fungi. All the extracts exhibited greater antifungal activity against Dermatophytes than the yeasts. The highest inhibitory effect of cold ethanolic extract reached to 96.92% at 20 mg/ml concentrations for *T. mentagrophytes* and *T. rubrum*, and 92.30% for *T. verrucosum*. In yeasts, the rate of inhibitory effect showed that with 200 mg/ml of cold ethanolic extract, the inhibitory rate of *C. albicans* and *C. neoformans* were 83% and 84% respectively.

This study, therefore, suggest that the green tea could have strong biocidal substance against Dermatophytes and some pathogenic yeasts, and therefore, may have the potential effective role in the treatment of human and animal dermatophytosis when used as ointment preparation.

دراسة التأثير التثبيطي لمستخلصات الشاي الأخضر على نمو بعض الفطريات الجلدية

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الخلاصة

أجري اختبار تأثير الشاي الأخضر من نوع *Camellia sinensis* كمضاد فطري لبعض الفطريات الجلدية مثل *Trichophyton mentagrophyte*, *Trichophyton verrucosum*, *Trichophyton rubrum* كذلك ضد بعض أنواع الخمائر الممرضة مثل *Candida albicans*, *Cryptococcus neoformans* حيث استخدمت طريقة تنقيع أوراق الشاي الأخضر بالكحول البارد والحر وكذلك الماء لغرض استخلاص المادة الفعالة منه. ودرست حساسية الشاي الأخضر كمضاد فطري من خلال تخفيف الوسط الزرعي بالمستخلصات المستخدمة. أظهرت هذه الدراسة ان المستخلص الكحولي البارد كان الأكثر فعالية ضد الفطريات المذكورة ثم يليه المستخلص الكحولي الحار أما المستخلص المائي فكان الأقل تأثيراً. لوحظ ان أعلى نسبة تثبيط للمستخلص الكحولي البارد لكل من *T. mentagrophyte* و *T. rubrum* كانت 96.92 % عند تركيز 20 ملغم/مل أما فطر *T. verrucosum* فقد وصلت نسبة التثبيط إلى 92.3% أما بالنسبة لخميرتي مثل *Candida albicans*

و *Cryptococcus neoformans* فقد لوحظ ان نسبة التثبيط كانت 83% و 84% على التوالي عند تركيز 200 ملغم/مل للمستخلص الكحولي البارد ولهذا اقترحت هذه الدراسة استخدام الشاي الأخضر لاحتوائه على مواد ذات فعالية بايولوجية قوية ضد الفطريات الجلدية وبعض الخمائر الممرضة وبالتالي إمكانية استخدامها كمراهم في علاج الإصابات الفطرية الجلدية في كل من الإنسان والحيوان.

Introduction

The use of plants for medicinal purposes permits the introduction of antibiotic and other modern drugs (1). The potency of herbal remedies soon became an issue of dispute due to lack of qualitative identification of their bioactive components (2). The search for more potent chemotherapeutic agents led to discovery and development of antibiotics (3). However, as years passed several microorganisms developed resistance, to these antibiotics thereby rendering them important and otherwise useless (4).

Overtime, the economy of producing these antibiotics and subsequent cost of acquiring medications was fast getting out of the reach of common man. In recent time, some of these antibiotics have been found to exhibit neurotoxic effects while a few others cause severe liver damage and bone marrow depression. All these factors led to the re-birth of intensive search for natural products from plants which contain active ingredients of medicinal values.

Green tea considered one of these important plants, It is originated from China and other Asian Countries which mainly produced from *Camellia sinensis* var *sinensis* has a too high content of polyphenols. Also it has been considered a medicine and healthful beverage since ancient time. This type of tea is produced by drying and steaming of fresh leaves to inactivate polyphenol oxidase and thus, non-oxidation occur (5).

However, it contains the catechins which have a wide range of strong antioxidants potential and possess antimutagenic, anticarcinogenic, antidiabetic and anti-inflammatory properties (6,7,8). In addition, the mineral compounds such as Fluoride and manganese are responsible for digestive tract function while the organic compound affects on activity of vision, skin, and cardiovascular system (9).

Further more, green tea leaves contain two main components which act upon human health: Caffeine and Theophylline, the first one acts mainly upon the central nervous system stimulant and the second one induces vasodilator and bronchodilator effects and a much higher diuretic effect than caffeine (10).

Large number of studies revealed that green tea catechins have antibacterial and antiviral activity by its effectiveness against any type of diarrhea and typhoid, also it inhibits the reproduction and growth of many bacteria, which some types of *Salmonella*, *Clostridium*, *Bacillus*, *Helicobacter pylori*, and *Staphylococcus aureus* (11). Regarding its antiviral action, it affects against the Influenza virus, especially in its earliest stage, as well as against Herpes simplex virus and Adenoviral infection. But the studies on its effect as antimycotic infection particularly antidermatophytic infections are very rare (12,13). Despite of wide-spreading of dermatophytosis which results in huge economical loss in animal products, treatment cost, in addition to increase rate of human infection, so the aim of this study is to demonstrate the capacity of many types of green tea extracts to kill or inhibit some types of dermatophytes and some pathogenic yeasts by comparison its effect with antimycotic.

Materials and Methods

- **Organisms:** The fungi used in this study were *T. mentagrophytes*, *T. verrucosum*, *T. rubrum*, *Candida albicans*, and *Cryptococcus neoformans*, isolated from patients with Dermatophytosis and diagnosed in Department of Microbiology, College of Veterinary Medicine. These strains were identified based on colony and microscopic morphology,

urease test, hair perforation test, germ tube test and ability to pigment production on Corn Meal Agar (CMA) plus 2% dextrose.

- Preparation of green tea extracts:

1. **Preparation of cold ethanolic extract:** Green tea leaves were obtained after imported from other country then pulverized into fine powder by using electric blender. Extraction was done with cold ethanol. Forty grams of powdered sample were added to 250 ml of 70% ethanol in flask for 24 hrs on magnetic stirrer in room temperature then the mixture was precipitated by centrifuge at 3000 rpm/15 min, after that the supernatant was collected and further filtered through filter paper Wattman No.1, the filtrate were evaporated to semi-solid mass. The dry extract were later reconstituted with their respective extractant (ethanol) to give a concentration of 200 mg/ml for antimicrobial activity which considered as a stock solution of extract which filtrated through 0.22 μ m Millipore and different concentrations of extracts (20, 10 and 5 mg/ml) were carried out to study the effectiveness of extract against Dermatophytes with plate of Sabouraud dextrose agar (SDA) free extract as control (14).
2. **Preparation of hot ethanolic extract:** This type of extract was carried out by taking 40 gm of powder and put in thumble of soxhlet apparatus. Then 250 ml of 70% ethanol was added to the extracted flask and the process was continued for 3 hrs at 60 °C then the mixture was filtered through Wattman No.1 . The other steps was similar to cold ethanolic extract (15).
3. **Preparation of aqueous extract:** For aqueous extract preparation, the same steps in the preparation of cold ethanolic extract was followed except of using distilled water instead of alcohol.

The radial growth was measured, and the rate of growth inhibition was calculated by using specific ruler (16).

- Effect of extracts on some yeasts:

The agar-well diffusion method was used (17) The agar plates wells were inoculated with 20 μ L of yeast suspension that contain (1×10^8 yeast/ml) via sterile swabs and it left at room temperature for 30 – 60 min to dry. Plugs were made at 6 mm by sterile cork porer, then different concentrations of extracts were prepared (200, 100, 50, 25 and 10 mg/ml) and were added 0.1 ml of each of them in each well with sterile distilled water in one of the well as control. After that the plates were inoculated at 37 °C for 24-48 hrs. The diameter of clear zone of inhibition was measured.

- Preparation of 1% clotrimazol as standared control:

Clotrimazol was used in this study to compare its effect against fungi with green tea extract effects. This was done by dissolving 50 mg of clotrimazol in 5 ml of organic dissolvent (Dimethyl sulphoxide 100%) to obtain of final concentration of 10 mg/ml (17).

Results

Effect of green tea extracts on the radial growth of some strains of dermatophytes are listed in (Tables 1, 2, 3), illustrated in (Fig 1), and the effect of these extracts on some pathogenic yeasts as zones of inhibition are listed in (Tables 4, 5).

This study found that the cold ethanolic extract was the most effective one, followed by the hot ethanolic extract while the aqueous extract was the least effective against all the test fungi.

Generally, there was a marked reduction in viability of all the test fungi with increased concentration. There was 96.92% loss of viability of *T.mentagrophytes*, *T.rubrum* and 92.30% of *T.verrucosum* at 20 mg/ml concentration of cold ethanolic extract when compared with standard antifungal (clotrimazol), and 83% and 84% of *C.*

albicans and *C.neoformans* at 200 mg/ml of cold ethanolic extract. Furthermore, it was shown that the *T.rubrum* ranked as the highest susceptibility against all types of green tea extracts, followed by *T.mentagrophytes* and the *T.verrucosum* ranked as the lowest effect degree through dermatophytes.

Table (1) Effect of cold ethanolic extract of green tea on the growth of some fungal isolates colony (mm)

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|-------------------|---------------------------------------|---------------------|-----------------|
| | <i>T.mentagrophytes</i> | <i>T.verrucosum</i> | <i>T.rubrum</i> |
| 0 (Control) | 65 | 19 | 73 |
| 5 | 60 | 14 | 30 |
| 10 | 50 | 10 | 20 |
| 20 | 2 | 5 | 2 |
| 1.25(Clotrimazol) | 0 | 2 | 1 |

Table (2) Effect of hot ethanolic extract of green tea on the growth of some fungal isolates colony (mm)

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|-------------------|---------------------------------------|---------------------|-----------------|
| | <i>T.mentagrophytes</i> | <i>T.verrucosum</i> | <i>T.rubrum</i> |
| 0 (Control) | 65 | 19 | 73 |
| 5 | 64 | 17 | 50 |
| 10 | 59 | 14 | 38 |
| 20 | 15 | 10 | 4 |
| 1.25(Clotrimazol) | 0 | 2 | 1 |

Table (3) Effect of aqueous extract of green tea on the growth of some fungal isolates colony (mm)

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|-------------------|---------------------------------------|---------------------|-----------------|
| | <i>T.mentagrophytes</i> | <i>T.verrucosum</i> | <i>T.rubrum</i> |
| 0(Control) | 65 | 19 | 73 |
| 5 | 65 | 19 | 65 |
| 10 | 60 | 15 | 60 |
| 20 | 20 | 13 | 18 |
| 1.25(Clotrimazol) | 0 | 2 | 1 |

Table (4) Effect of different extract concentrations on the growth inhibition of *Candida albicans* (mm)

| Conc. (mg/ml) | Mean of diameter of fungal colony(mm) | | |
|------------------|---------------------------------------|-----------------------|-----------------|
| | Cold ethanolic extract | Hot ethanolic extract | Aqueous extract |
| 0(Control) | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 50 | 11 | 2 | 0 |
| 100 | 15 | 12 | 2 |
| 200 | 17 | 15 | 10 |

Table (5) Effect of different extract concentrations on the diameter inhibition of *C.neoformans* (mm)

| Conc. (mg/ml) | Mean of diameter inhibition of extract(mm) | | |
|------------------|--|-----------------------|-----------------|
| | Cold ethanolic extract | Hot ethanolic extract | Aqueous extract |
| 0(Control) | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 50 | 10 | 0 | 0 |
| 100 | 13 | 11 | 6 |
| 200 | 16 | 15 | 10 |

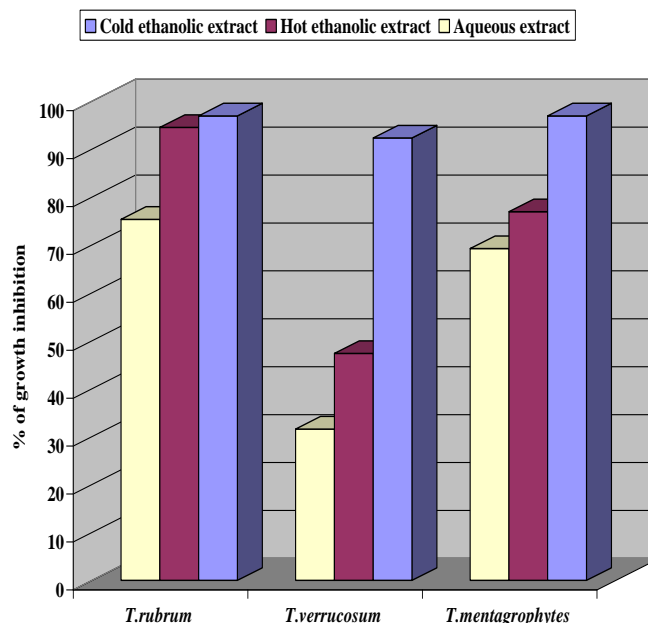


Fig (1) Effect of Green tea extracts at 20 mg/ml on some strains of dermatophytes

Discussion

Tea is the most consumed drink in the world after water. Green tea has been represented a healthful beverage since ancient time (18) mentioned the chemical composition of green tea is complex: protein (15-20% dry weight), aminoacid (1-4% dry weight), carbohydrates (5-7% dry weight), lipid, vitamins (B, C, E), pigment, volatile compound, mineral and trace elements (5% dry weight) .But the most intresting group of this type of tea leaf components is polyphenols. So, this tea can be considered as an important dietary source of polyphenols, particularly flavonoids. These flavonoids are phenol derivatives synthesized in substantial amount (0.5-1.5%) as studied by (19).

Many studies conducted over the last 20 years have shown that the green tea plyphenolic catechine, in particular (-)-epigallocatechin-3-gallate (EGCG) represent approximately 59% of total catechines, (-)-epigallocatechin (EGC) 19%, (-)-epicatechin-3-gallate (ECG) 13.6% and (-)-epicatechin (EC) 6.4%. This study shows the inhibitory effect of green tea extracts on some strains of dermatophytes (*Trichophyton* spp.) due to the catechin attached the cell membrane and caused lysis of the conidia and hyphae, these findings agreed with (20) and (21) when they used electron microscopy.

In contrast with (22) who reported that 2.5% of Black tea extract completely inhibited the growth of *T. mentagrophytes* and *T. rubrum*. However, even at 10% concentration, this extract did not inhibit the growth of *C. albicans* or *C. neoformans*.

In this study demonstrated the inhibitory effect of green tea extracts suppress the growth of moulds and yeasts but to different extent depending on extract concentrations, and the fungicidal effect could be due to EGCG, EGC and GC.

Although (23) highlighted that the EGCG could inhibit ergosterol synthesis by disturbing folic acid metabolism in *Candida albicans* which have been proved by (24).

In the present study, the cold ethanolic extract exerted the greatest inhibitory activity against the tested fungi followed by hot ethanolic extract, while aqueous extract exhibited the least, and this due to activity of ethanol to dissolve multivariable compounds either polar or non-polar as mentioned by (25) which may be responsible for the greater antifungal efficacy than water.

The inhibitory effect of tea depends upon type of tea, preparation of the extracts method and its concentration and tested microbes, However, the biological activity of the tea increased with high concentration of extract.

In a study to (26) who record the sensitivity of 10 different wood-rotting fungi towards eight samples of tea and two samples of coffee, they discovered that the green tea caused the maximum growth inhibition and it was 100% in case of *Phanerochaete chrysosporium* and *Sporotrichum pulverulentum* While the effect of hot ethanolic extract could be due to thermostability of some bioactive chemical constituents which might have been enhanced by the possibility of an increase in the solubility of active ingredients of plant material in hot alcohol making more constituents available in the resulting extract.

However, this work has shown that ethanol is the extractant of choice because the bioactive substances in green tea tested are less soluble in water than in ethanol.

Therefore, using appropriate extractants could be purified and manufactured as antiseptic agent (as ointment) for the treatment of skin infections caused by these groups of fungi. Also these extracts could be combined with antimycotics that may be beneficial and may contribute and increase the effective medical treatment of these fungi.

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Sex Chromatin Picture in Sharabi Cows in Iraq

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Abstract

The incidence of sex chromatin and its various shapes in polymorph nuclear leucocytes (PMNL) was investigated in Iraqi cows (Sharabi). A total of 72 animals were included in the study (15 heifers and 57 adult cows, both fertile and those suffering from reproductive problems). Results indicated, that sex chromatin incidence in Sharabi heifers (6 month old) was 6.9% distributed in 4 shapes (Drumstick 2.64%, Small club 0.28%, Tear drop 1.42, Sessile nodule 2.57%). In the fertile adult cows (4-6 years old) the incidence was 8.7% which was significantly ($P < 0.05$) higher than in heifers, distributed in 4 shapes: Drumstick 4.1%, small club 0.5, Tear drop 0.9, Sessile nodule 3.2%. The % of Drumstick and sessile nodule was significantly ($P < 0.05$) higher than in heifers. Concerning infertile animals the incidence of sex chromatin decreased significantly ($P < 0.05$) in animals suffering from abortion, still birth & repeat breeder than the fertile animals. The distribution of various shapes of sex chromatin also decreased significantly in all these animals as compared to fertile animals. Key Words: Sex chromatin, Polymorph nuclear leucocyte, reproductive problems, cows.

صورة الصبغين الجنسي في الأبقار الشرايبية في العراق

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الخلاصة

الهدف من هذه الدراسة قياس نسبة تواجد الصبغين الجنسي وأشكاله المختلفة في خلايا العدلات للأبقار الشرايبية. أجريت الدراسة على 72 بقرة بواقع 15 بقرة بكر و 57 بقرة بالغة صحيحة وتعاني مشاكل تناسلية. أوضحت النتائج بأن نسبة تواجد الصبغين الجنسي في الأبقار الشرايبية الأباكير (عمر 6 شهر) كانت 6.9 % موزعة على أربعة أشكال عصا الطبال 2.64 %، بروز بدون ساق 0.28 %، دمعة العين 1.42 %، الهراوي 2.57 %. في الأبقار البالغة (4 - 6 سنوات) كانت نسبة تواجد الصبغين الجنسي 8.7 % والتي أظهرت اختلافا معنويا ($P > 0.05$) عنها الأباكير وموزعة كالآتي: عصا الطبال 4.1 %، بروز بدون ساق 0.5 %، الهراوي 3.2 %، ودمعة العين 0.9 %.

لقد أظهرت نسبة تواجد شكل الهراوي وعصا الطبال اختلافا معنويا بمستوى معنوية ($P > 0.05$) في الأبقار البالغة عنها الإناث الأباكير.

وفيما يتعلق بالإناث التي تعاني مشاكل تناسلية انخفضت نسبته تواجد الصبغين الجنسي معنويا تحت مستوى ($P > 0.05$) في الحيوانات التي تعاني من الإجهاض والأجنة الميتة والصرف المتكرر عنها في الحيوانات السليمة. وانخفضت نسبة تواجد الصبغين الجنسي بأشكاله الأربعة في الأبقار التي تعاني مشاكل تناسلية بالمقارنة مع الحيوانات السليمة.

Introduction

It has been reported that sex chromatin abnormalities in bovine is associated with impaired reproductive efficiency (1,2,3,4,5). Moreover, cows suffering from anestrus showed; absence of sex chromatin (drumstick) in their polymorph nuclear leucocyte (6). Cows in Iraq (lopl or imported) suffer from several reproductive problems. In view of the fact that no study has been reported on the sex chromatin pattern in cows in Iraq, the present investigation was conducted to study sex chromatin in a local cows, Sharabi, in both fertile and infertile animals.

Material and Methods

The study was conducted on a total of 72, Sharabi, (local breed) cows both, heifers 6 months old (15) and adult cows, 4-6 years old, (57). The animals were raised in Al-Rashidya field station for animal production, Mousl (IRAQ). They were kept under a fairly good management and both concentrated & green food were available along with water *ad libitum*.

Blood samples were collected from the Jugular vein using heparinized vacutainer tubes after that, they were centrifuged and the buffy coat was aspirated. Blood smears were prepared from the aspirated portion, stained with Wright Giemsa stain (7) before being examined under the microscope using oil immersion. Sex chromatin of different shapes were counted in 300 polymorph nuclear leucocytes animal, the vertical and horizontal axis were determined together with their area (SCA) and nuclear area. Results were analyzed using computerized general linear model.

Results

- **Sex chromatin incidence in heifers:** It is shown in table 1, that percentage of sex chromatin occurrence in heifers is 6.9% distributed in 4 shapes . drum stick, small club, tear drop, and Sessile nodule with the following percentages for various shapes respectively : 2.64, 0.28, 1.42 and 2.57.
- **Sex chromatin incidence in adult cows:** Incidence of sex chromatin in adult cows was: 8.7%. This value is significantly ($P<0.05$) higher than in heifers (Table 1). Concerning various shapes of the sex chromatin no significant change was noticed between heifers and adult animals.
- **Sex chromatin dimensions and animal age:** Regardless of shape, the vertical & horizontal axis of the sex chromatin showed no significant difference between heifers and adult cows studies at different ages (Table 2). Nuclear area (NA), decreased significantly ($P<0.05$) at 4 years of age as compared to 5 and 6 years of age (Table 2). Ratio of NA/SCA revealed a significant ($P<0.05$) decrease at 0.5 and 4 years of age as compared to all other ages. SCA/NA decreased significantly ($P<0.05$) at 5 and 6 years of age as compared to 0.5 and 4 years of age (Table 2).
- **Sex chromatin % in adult cows:** The % of SC in fertile animals at all ages showed a significant ($P<0.05$) increase over infertile animals suffering from abortion, still birth and repeat breeder (Table 3). A similar trend was also seen when various shapes of SC was studied. The percentage of SC of drumstick shape and sessile nodules decreased significantly ($P<0.05$) in the infertile compared to the fertile cows.
- **Sex chromatin dimensions in adult cows:** No significant difference was seen in both the vertical and horizontal axis length in fertile as compared to infertile cows (Table 4). However SCA decreased significantly ($P<0.05$) in animals having dead birth in comparison with both fertile and other infertile cows. In animals suffering from abortion, NA increased significantly ($P<0.05$) over the fertile animals. The

ratio of NA/SCA decreased significantly ($P<0.05$) in animals suffering from abortion as compared with those having dead birth. The other ratio (SCA/NA) showed no significant difference neither when fertile animals are compared with the infertile nor when infertile animals were compared with each other (Table 4)

Table (1) Percentage of sex chromatin in polymorph nuclear leucocytes in Sharabi cows at different ages

| Animal age (year) | Sex chromatin % | Drumstick % | Small club % | Tear drop % | Sessile nodule % |
|--|-------------------|-------------|--------------|-------------|------------------|
| Heifers (0.5) | B 6.9 | 2:64 | 0.58 | 1.12 | A 2.57 |
| Adult (4,5,6 years) 4 years 5 years 6 years | 7.45 A 7.96 | 3.35 3.66 | 0.37 0.3 | 0.87 1 | 2.86 A 3 |
| | AB 7.4 | 3.2 | 0.4 | 1 | A 2.79 |
| | AB 7 | 3.2 | 0.4 | 0.6 | A 2.8 |

- Values with different letters indicate a significant difference ($P<0.05$)

Table (2) Changes in sex chromatin dimensions , nuclear area (NA) , sex chromatin area (SCA) and their ratios in polymorph leucocytes of Sharabi cows at different ages

| Animal age (year) | Vertical axis (m n) | Horizontal axis (m fj.) | Sex chromatin area (m) | Nuclear area(m Ji) | NA/SCA | SCA/NA |
|-------------------|---------------------|-------------------------|------------------------|--------------------|-----------------|----------------|
| 0.5 | A 1.35±0.43 | A 1.3310.24 | A 1.3410.64 | A 30.4311.62 | A 24.4213.06 | A 4.0710.45 |
| 4 | A 1.1910.29 | A 1.2010.24 | A 1.1510.43 | B 26.2612.59 | B 24.414.10 | A 4.8210.55 |
| 5 | A 1.27±0.37 | A 1.2110.24 | A 1.2410.51 | A 31.6911.79 | A 29.8113.71 | B 3.0110.48 |
| 6 | A 1.2110.28 | A 1.1810.27 | A 1.1710.53 | A 31.6512.62 | A 30.7515.20 | B 2.9210.28 |

- Values carrying different letters indicate a significant difference ($P<0.05$)

Table (3) Sex chromatin percentages in the polymorph nuclear leucocytes of Sharabi cows both fertile and infertile

| Animal state | Sex chromatin % | Drumstick % | Small club % | Tear drop % | Sessile nodule % |
|---|-----------------|-------------|--------------|-------------|------------------|
| Fertile | A 8.7 | A 4.1 | A 0.5 | A 0.9 | A 3.2 |
| Infertile: Aborted Dead birth Repeat breeder | B 6.0 | B 2.5 | A 0.7 | A 0.8 | B 2.0 |
| | B 5.66 | B 2.33 | A 0.5 | A 0.93 | B 1.90 |
| | B 5.66 | B 2.35 | A 0.65 | A 0.66 | B 1.8 |

Values with different letters indicate a significant difference ($P<0.05$).

Table (4) Changes in the dimensions of sex chromatin in fertile & infertile adult Sharabi cows including nuclear area (NA) ,sex chromatin area (SCA) and their ratios

| Animal age (year) | Vertical axis (m ^) | Horizontal axis (m n) | Sex chromatin area (m n) | Nuclear area (m ^i) | NA/SCA | SCA/NA |
|-----------------------|---------------------|-----------------------|--------------------------|---------------------|------------------|------------------|
| Fertile | A 1.2510.35 | A 1.2310.23 | A 1.2610.61 | B 30.7712.10 | AB 28.8314.18 | - A 4.3112.17 |
| Aborted | A 1.11 ±(>.27 | A 1.1810.33 | A . 1.0610.35 | A 36.0011 80 | B 25.1514.54 | A 3.1 IK). 55 |
| Dead birth | A 1.1210.24 | A 1.1310.32 | B 1.0010.34 | A 32.9212.33 | A 32.9214.69 | A 3.1510.29 |
| Repeat breeder | A 1.2010.38 | A 1.2510.24 | A 1.2010.55 | AB 32.4211.72 | AB 28.5613.59 | A 4.1310.91 |

Values with different letters indicate a significant difference (P<0.05)

Discussion

The significant increase in the incidence of sex chromatin in polymorph nuclear leucocytes with the advancement of age (from 0.5-4 year) agrees with that reported in the literature. It has been found that there is a steady increase in bovine sex chromatin with advancement of age (8,4). A similar observation has been reported in the sheep and goat (9,10). The reason behind the significant

decrease seen in nuclear area (NA) in cows 4 years old in comparison with subsequent ages is not known, however, it has been reported that cells having smaller nuclei tend to have a higher incidence of sex chromatin (11,12), a trend similar to what we have obtained in our study (Table 2). This change in nuclear area could have influenced both NA/SCA ratio (decreased significantly) and SCA/NA ratio (increased significantly) as compared to subsequent age (Table 2).

The significant decrease in sex chromatin % in infertile cows agreed with the result of other worker concerning other breeds of cows (12,13). A similar trend has also been reported in sheep & goats (10). It has been suggested that female animals suffering from certain reproductive problems have a delayed DNA synthesis from the inactivated chromosomes (12,14). More over, it has been reported that abnormal hormonal levels associated with certain reproductive problems may influence sex chromatin percentage and dimensions.

The significant decrease in drumstick % observed in infertile cows may be the main cause of the significant decrease in the total % of sex chromatin of these animals.

The Changes seen in nuclear area (NA) and NA/SCA ratio in infertile cows may be attributed to changes in sex chromatin %, which seem to have a negative relationship with nuclear area.

It was concluded from the study that the SC play arole in reproductive process and indicate the need for further studies to clarify changes in sex chromatin under various hormonal levels or in different reproductive pathological conditions.

Acknowledgment

We acknowledge the help provided by the animal production field station, Mousl, IRAQ (Ministry of Agriculture) for facilitating the performance of this research in the station.

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Histopathology of the liver affected With Aflatoxins in broiler chicks**S. A. Lafi*, N. A.Taha** and S. M.H. Al-Genabi******College of Medicine\ University of Anbar****** College of Dentistry\ University of Anbar****Abstract**

The study was conducted to show the effects of aflatoxine on histopathological picture of the liver of broiler chicks. Twenty five rose broiler chicks had been claimed 42 days of age reared in broiler form in Heat/ Al- Anbar province. The criteria of diagnosis of aflatoxicosis depends upon, clinical signs, mortality rate and postmortem exam, of the cases. The liver of all birds were inspected grossly, after cervical dislocation and necropsied.

Liver fragments were collected in 10% neutral buffered formalin. Tissue section 5 Mm thick were stained with hematoxylin and eosin and used for histopathological evaluation. Gross inspection of the liver showed enlargement congestion with pale patches. The histopathological changes in liver tissues include hyper plasia, congestion, necrosis, cirrhosis accumulation of RBC and inflammatory cells around the central vein.

دراسة نسيجية مرضية لكبد فروج اللحم المصابة بسموم الافلاتوكسين**شهاب أحمد لافي*، نجم الدين عبد الله طه** وسميعة مجبل حمد الجنابي****** كلية الطب/ جامعة الأنبار****** كلية طب الأسنان/ جامعة الأنبار****الخلاصة**

الغرض من الدراسة معرفة تأثير سموم الافلاتوكسين على الصورة النسيجية المرضية لكبد فروج اللحم أجريت الدراسة على 25 فروجه من اللحم من سلالة الروز في حقل في الأنبار ويعمر 42 يوم شخصت الحالات بإصابتها بالافلاتوكسين اعتماداً على العلامات السريرية والتشريح المرضي ونسبة الهلاكات. تم فحص الكبد بعد إجراء التشريح المرضي بعد قتل الطير. جمعت عينات من الكبد ووضعت في محلول متعادل من الفورمالين 10% قطعت النماذج نسيجياً ووضعت بالهيماتوكسلين ايوسين. لغرض قراءة التغيرات النسيجية المرضية. لوحظ في الفحص العياني للكبد وتضخم في الكبد ومع وجود احتقان مع شحوب في لونه. أظهرت التغيرات النسيجية، جود فرط تنسج في خلايا الكبد، احتقان، تنكز مع حدوث تلف في النسيج الكبدي. لوحظ تجمع الخلايا الحمراء والتهاب حول الأوردة المركزية.

Introduction

Aflatoxins are produced by fungi of genus *Aspergillus*, particularly *A.flavous*, *A.parasiticus* and *A.nomius* (1). Seventeen metabolites have been identified as aflatoxins , with aflatoxin B1 (AFB1) being the most commonly found metabolite in cereals and the one that exhibits the highest toxigenic effects (2). Aflatoxins causes a great biochemical changes includes, energy carbohydrates and lipids, nucleic acid and protein metabolism (3). Their biological effects include carcinogenicity, mutagenicity, teratogenicity and hepatotoxicity (4). Aflatoxins are afrequent problem for poultry production resulting in poor bird performance (2) , which is caused by several factors including reduced activity of pancreatic enzymes, decreased concentration of bile (4), increased incidence of leg problems, injury to the sciatic nerve (Lessons and summers , 1988), and antagonism in the metabolism of vitamins , proteins and amino acids , lipids , carbohydrates and damage (4,5,6).

Aflatoxin causes a variety of effects in poultry, including a decrease in body weight gain and efficiency of feed utilization. In poultry AFB1 is associated with liver damage, poor performance and immunosuppression. Liver characteristically show biliary and nodular hyperplasia and are pale and enlarged as aresult of aflatoxicosis (7,8). The study was aimed to show the effect of aflatoxicosis on histopathological picture of the liver in broiler chicks.

Material and Methods

Twenty five rose broiler chicks had been claimed at 42 days of age reared in abroiler farm in Heat/ Al- Anbar province. The criteria of diagnosis of aflatoxicosis depends upon, clinical signs, mortality rate and postmortem exam of the cases. The liver of all birds were inspected grossly. After cervical dislocation and necropsied liver fragments were collected in 10 percent neutral buffered formalin. Tissue section 5Mm thick were stained with hematoxylin and eosin and used for histopathological evaluation (9).

Results

The clinical signs appeared on the chicks include; reduction in feed consumption, depression, ruffled feathers, Closed eye, Stunted growth, purple discoloration of feet and leg and lameness ataxia, convulsions, opisthotonus preceded death. At necropsy, liver and kidneys were enlarged and pale. Some cases had hydropericardium and ascites, shrunken firm nodular liver, bil distended gall bladder and may be hemorrhagic. The histopathology of livers of broiler chicks stained with hematoxylin and eosin showed multifocal and varied cytoplasmic vaculation with perilobular location. Some hepatocytes have small pyknotic nuclei, very mild infiltration of polymophonuclear leukocytes is also present (Fig. 1). There are hepatocellular degeneration and swelling due to hydropic degeneration and fatty changes.

The bile ducts showed hyperplasia and hetrophilic infiltration (Fig.2). Bile duct proliferation and mononuclear infiltration in the portal triad with mild hydropic degeneration of hepatocytes. There is also hemorrhage and centrilobular to massive hepatocellular necrosis (Fig. 3). There is a proliferation of fibroblast with fibrous tissue formation around blood vesssels which extend to hepatic tissue (Fig. 4).

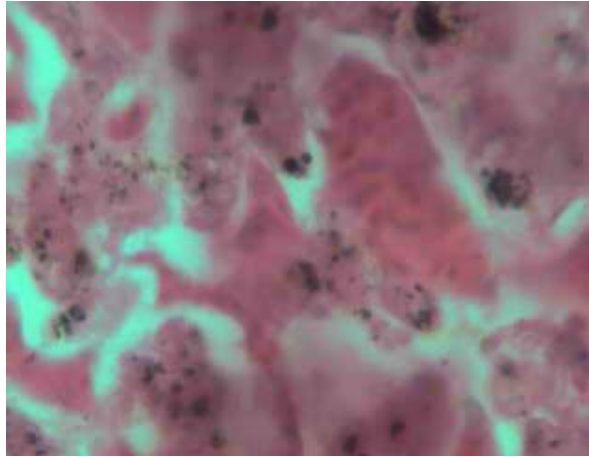


Fig (1) Numerous fat containing vacuoles of widely varying size in liver

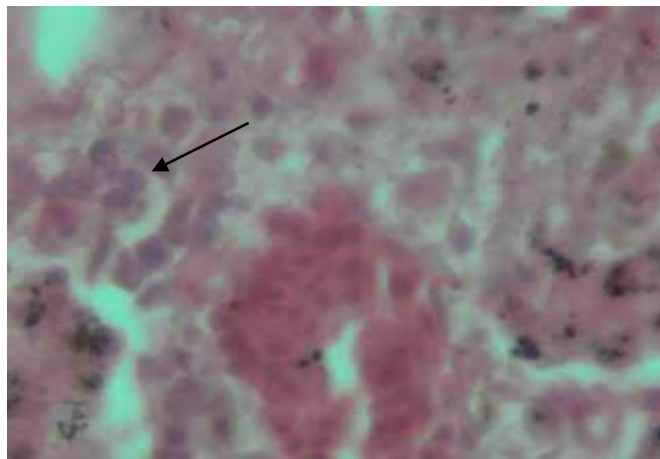


Fig (2) Swelling due to hydropic degeneration in liver

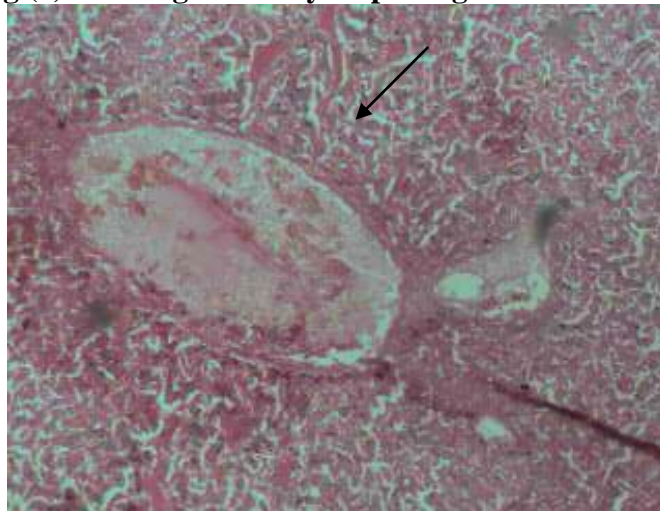


Fig (3) Bile duct proliferation and mononuclear infiltration of PMNs. Hemorrhage and centrilobular to massive hepatocellular necrosis

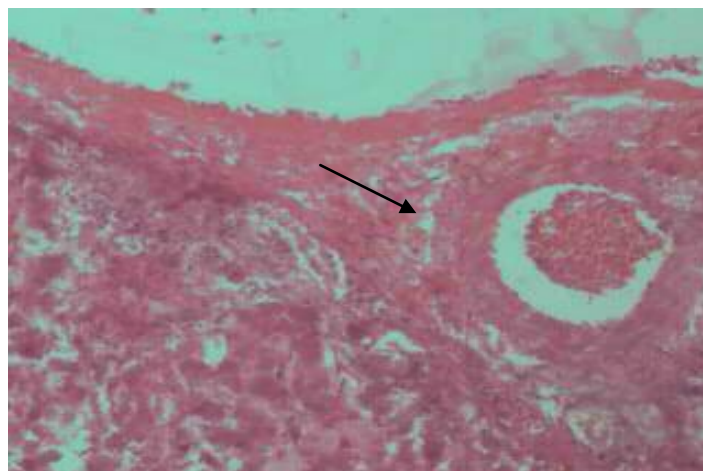


Fig (4) Fibrosis. Fibrous tissue around the blood vessel and extend to the hepatic tissue

Discussion

Aflatoxicosis is an important to the poultry industry because of their toxicity and frequency of occurrence in feed stuffs. It produces great economic losses affecting ducklings, broilers, layers, turkeys and quail. Aflatoxin impairs all important productive parameters including weight gain, feed intake, feed conversion efficiency (10,11,12). The symptoms noticed on affected birds, which includes; anemia, paralysis and lameness, ruffled feathers, closed eye, stunted growth Ataxia and convulsions is similarly observed by other workers (10,11,13,14).

The study revealed the harmful effect of Aflatoxins exposure to broiler liver tissue. These effects include hyperplasia, necrosis, cirrhosis and fibrosis of the liver in infected chicks. This is in agreement with the observation of many research workers (15, 16, 17). This might be due to damage caused by aflatoxin to liver tissue and bile duct (18). The most characteristic gross lesions appeared in the livers which were enlarged, pale yellow to grayish brown and had a prominent reticular pattern. petechial hemorrhages were observed on the surface of some livers, gall bladders were enlarged and bile duct distended (12,19). The liver, spleen and kidney were increased in size, whereas the bursa of Fabricius decreased (10,12). The histopathological picture observed in our study had been previously noticed by other research workers (13, 19,20). It was concluded from this study that Aflatoxins caused a great liver damage and may require to a new approach to treat the aflatoxin contaminated feed stuff.

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