Teratogenic effects of aflatoxin in Rabbits (*Oryctolagus cuniculus*)

S.M. El-Nahla[1]; H.M. Imam[1]; E.A. Moussa[1]; A.M. Ibrahim[2] and A.R. Ghanam[1].

[1] Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt;
[2] Department of pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt;

With 18 figures Received June, accepted for publication August 2013

Abstract.

This study was conducted to determine the teratogenic effects of aflatoxin B1 (AFB1) in balady rabbits. The female animals divided into two groups control and treated, each group contained three dams. A dose of 0.05mg /kg/day AFB1 was administered by gastric intubation to pregnant rabbits on the 6^{th}–18^{th} day of pregnancy. The fetuses were obtained through the uterine incision at 29^{th} day of gestation. The lengths and weights of the fetuses as well as absolute organs weights were measured, revealing the statistically significant differences between the two groups ($p<0.001$).The observed gross anomalies included wrinkled skin, enlarged eye socket and microphthalmic eyes. The heart of treated group showed reduction in size with wide ventricular lumen and shallow inter ventricular groove. The characteristic fetal histopathological findings were vaccuolation and distortion of hepatic cord pattern. The renal tubular epithelium was vacuolated and their lumen were occluded with casts. The eye lids revealed less number of hair follicles. Regarding, the skeletal anomalies there were incomplete ossification in some of the skull bones, the laminae of the vertebral arches throughout the vertebral column remain cartilaginous. The sternum was incompletely ossified. The 2^{nd} phalanx, carpus, extremities of metacarpi had no cartilaginous drafts. The central and distal tarsal rows as well as extremities of metatarsi remained cartilaginous..

Keywords: Aflatoxin B1 (AFB1), Rabbits, teratogenicity, Ossification, histopathology.

Introduction:

Mycotoxins are secondary metabolites produced by fungi. It causes potential threat to animal and human health. Among commonly occurring mycotoxins, aflatoxin B1 (AFB1) which gained immense importance due to its biological effects and widespread toxicity (Trail et al., 1995). AFB1 is one of stable mycotoxins commonly produced by toxigenic strains of Aspergillus, A. flavus, A. parasiticus and A. fumigatus, that are ubiquitous in hot/ humid conditions and natural contaminates of food and feed stuff (Hussein and Brasel, 2001; Diekman and Green, 1992). In,(1993) WHO-international agency for research and cancer classified AFB1 as Group 1 carcinogen. AFB1 is one of important food borne mycotxins and its ingestion correlated with high incidence of liver cancer in human (Trail et al., 1995).

Congenital malformations are structural anomalies which take place during embryogenesis. Several medicines and certain chemicals may cause malformations or permanent defects which may lead to death through passing fetal circulation, which is called (teratogenesis) (Brendel, Duhamel and Shepard (1989).

Development of embryo is affected by teratogens mostly during the process of organogenesis, which is recognized as the time period from the occurrence of the neural plaque to closure of the plate (Stanley and Bower, 1986and Vickers and Brackley, 2002). It begins usually on the 6^{th}–18^{th} days in rabbit (Petrere, et al., 1993 and Wangikara, et al. 2005) during this period, teratogenic agents can lead significant congenital anomalies.

AFB1 was recorded to be teratogenic in rats (butler and Wigglesworth, 1966, Sharma and Sahai, 1987; Mayura et al, 1998 and Wangikar et al, 2004b), mice (Dipaolo et al, 1967 and Arora and Frolen, 1981), hamsters (Schmidt and Panciera.1980) and rabbits (Wangikar et al, 2005). Rabbit is the most sensitive and suitable laboratory animal for studying the teratogenic potential of different chemicals (WHO, 1993).

The acute oral LD50 of AFB1 for rabbits was 0.3 mg/kg body weight (Clark, et al., 1980). While the dose of 0.1 mg/kg AFB1 body weight AFB1is the minimum teratogenic dose in rabbits that interfere with intrauterine development during 6^{th}-18^{th} days of gestation (Wangikar et al., 2005). However, the present work used a dose of 0.05 mg/kg AFB1 as a teratogenic because the dose of 0.1mg/kg AFB1 causes death to the dams at 14^{th} day of gestation.

However, the literature about the teratogenic study in rabbits are meager and the rabbit is the most sensitive species to mycotoxins and greatly similar to humans in early developmental patterns (Beaudion, et al., 2003) in addition to the extra-embryonic membranes of rabbits

are closely resemble to that of human (Foote and Carney, 2000)

Material and methods

The present work was carried out on (8) adult; apparently healthy balady rabbits sexually mature of both sexes (2 males &6 females) from 3-4 months old and weighing 2.5-3 kg. The animals were kept for two weeks for acclimatization with the housing conditions. The female rabbits in estrous were mated with males and considered day zero of conception. The pregnant animals were divided into two groups and each individual was kept in a separate cage. All animals were kept under a constant day/night cycle (12 h L/12 h D) for 29 days (pregnancy period). All animals received routinely ad libitum standard rabbit diet (18% crude protein containing granule pellet) tested free from any mycotoxins at Central Laboratory of Residue Analysis of Pesticides and Heavy metals in food, Agricultural Research Center, Ministry of Agricultural, EGYPT. Tap water was supplied ad libitum on a daily basis. On the 29th day of pregnancy, fetuses were taken by uterine incision after slaughter of the dams.

1. method of treatment

The **control group**: three animals were dosed by gastric intubation with a dose of 0.2 ml corn oil/kg/day from 6^{th}-18^{th} days of the pregnancy.

The **intoxicated group**: three animals were received AFB1 with a dose of 0.05mg/kg dissolved in corn oil 0.2 ml/kg/day from 6^{th}-18^{th} days of the pregnancy.

2. fetus preparation

All the collected fetuses were weighed separately and measured their crown rump length (CRL). The fetuses were carefully examined for gross morphology, visceral anomalies (liver, heart, kidney, brain and eye) as well as the skeleton.

3. Fetus staining for skeleton

Ten Fetuses from **the control group** and (10) fetuses **the intoxicated group** were randomly selected and fixed in absolute ethyl alcohol and stained with Alizarin Red-S-Alcian Blue to define the mineralized areas and the cartilage, respectively. The amount of mineralization was calculated as the length of stained portion of the bone by Alizarin Red-S.

The fetuses were firstly fixed in 95% ethyl alcohol for 7 days and subsequently put in pure acetone for degreasing for 3 days. Then, skin and internal organs were totally removed to achieve better staining results.

1- They were stained according to the method described by **Inouye,** (**1976**) and **Young**, **Phipps** and **Astroff**., (**2000**).
2- Then the specimen were transferred to transparency process using ascending series of glycerol and 1% aqueous solution after KOH after which they were preserved in 100% glycerin.

3- The stained preparations were carefully examined using Stereo-microscope (OPTIKA) to illustrate the different parts of the bones of both axial and appendicular skeleton.

4. Fetuses preparation for histopathological sections

For light microscopy, Serial sections were taken (liver, kidney and eye) of both control and experimental fetuses

Cross sections of (4-6) mm in thickness were obtained, stained with harries haematoxylin & eosin (H&E) as outlined by (Bancroft and Gamble, 2007).

5. Statistical analysis

The individual data on, relative organ weight, CRL and fetal weight were subjected to descriptive statistics (mean and standard deviation), followed by analysis of variance (ANOVA) and T- test according to (Argyrous, 2005) using statistical Package for Social Science (SPSS) software program version 16.0.and Excel Microsoft office program (2003). Statistical significance was accepted at $P < 0.05$.

Nomenclature used in this study was adapted according to Nomina Anatomica Veterinaria (2005) and Nomina Embryologica Veterinaria (2003) whenever possible.

Results.

1. Macromorphometric measurements and gross anatomical findings

There were no mortalities of any fetuses from intoxicated dams during the period of experiment.

The mean fetal weight of intoxicated and control fetuses was 28±1.29 and 54.28±8.26 respectively while, the mean crown rump length was 9.84±0.21 and 11.57± 0.47 respectively. They were significantly reduced compared with control one table (1).
The gross anomalies of treated fetuses were sever subcutaneous congestion at different regions including abdomen and head mainly in the eye region. In addition to, enlarged eye socket with microophthalmic eye and wrinkled skin fig (1). Also, there were small sized liver as well as cardiac defects including small sized heart with shallow inter-ventricular grooves and wider ventricular lumen compared to control group fig (2).

The mean absolute weights of different fetal organs (liver and gall bladder, stomach and intestine, heart and lungs and kidneys) of treated dams were significantly decreased compared to control fetuses at 29^{th} days as follow (**2.52 ±** 0.296 and 3.23 ± 0.23,, 1.84 ± 0.11 and 2.47 ± 0.11,, 1.69±0.11 and 1.89±0.09,, 0.247± 0.011 and 0.33±0.036) respectively fig (3) and table (1).

2. Fetal patho-morphological observations

The main histopathologic changes were mainly seen in the liver, kidney and the eye. The tissues sectioned from the control fetuses presented a normal histological picture while that in the treated group, the liver showed vaccuolation of hepatocytes , as it was evident by large clear vacuole occupies the cytoplasm and pushing the nucleus to the periphery. Some hepatocytes had pyknotic nucleus. Extra medullary hematopoiesis was subnormal fig (4). The renal tubular epithelium of some tubules showed granular vacuolated cytoplasm and prominent casts. Some lining cells were proliferated had vesicular nucleus, degeneration and atrophy of the glomeruli fig (5). The numbers of hair follicles in the eye lid dermis were fewer than that of the control group fig (6).

3. Skeletal anomalies

3.1. Skeleton axiale

Many of the skull bones developed through the process of intramembranous ossification, which does not involve a cartilaginous draft. While bones at the base of the skull including the occipital and basisphenoid bones developed through endochondral ossification.

- **The control group.**
  The Cranium showed incomplete ossification between parietal and frontal bones where the rostral fontanelle was small and still open and the closure of the sutures (sagittal suture between the two parietal bones, the coronal suture between the frontal and parietal bones, the interfrontal suture between the two frontal bones as well as the occipito-parietal suture between the parietal and occipital bones) were established but still not closed. While, the interfrontal, frontonasal and internasal sutures were well established and closed fig (7& 8). There was nearly complete ossification between basioccipital and basisphenoid bones as well as between the pre sphenoid and vomer bones while the basisphenoid and pre sphenoid bones were still connected by a plate of cartilage fig (9).
  Regarding the colunma vertebralis, the bodies and arches of all vertebrae of the control fetuses were markedly stained red due to their ossification. The transverse processes of the lumbar vertebrae were well formed fig (10)
  The ribs of the control fetuses were markedly stained red due to their complete ossification fig (11)
  The sternebrae of the control fetuses formed of clearly six bony segments completely ossified and stained red.

- **The intoxicated group (Aflatoxicated group).**
  The treated fetuses showed a wide membranous rostral fontanelle. All the developing sutures

(sagittal suture between the two parietal bones, the coronal suture between the frontal and parietal bones, the interfrontal suture between the two frontal bones, the occipito-parietal suture between the parietal and occipital bones, the interfrontal sutures between the two frontal bones, the frontonasal sutures between the frontal and nasal bones as well as the internasal suture between the two nasal bones) had smooth adjacent margins and separated from each others by membranes fig (12). The ventral surface of the skull of this group of fetuses showed cartilaginous drafts between basioccipital and basisphenoid bones, between the pre sphenoid and vomer bones as well as between the basisphenoid and pre sphenoid bones fig (13).

Regarding, columna vertebralis, the dosed fetuses with AFB1 showed blue stained laminae of the vertebrae because they remain cartilaginous and other parts of the vertebrae (bodies and pedicles) stained red fig (10)
The transverse processes of the lumbar vertebrae were reduced compared to the control one.

The ribs of treated fetus showing dark violet due to their incomplete ossification fig (11).

The sternebrae had five segments with faint red coloration due to delay in their ossification

3.2. Appendicular skeleton

The bones of the **for limb** (scapula, humerus and radius and ulna) of the control fetuses were grossly longer than those of the treated fetuses **fig** (14A &B)

In the **control group**, the distal extremity of the radius and ulna and proximal and distal extremities metacarpi as well as the carpus were still in the nature of a cartilage draft. While, the **treated group** showed blue coloration for both extremities of radius and ulna and their shafts showed violet color due to incomplete ossification of the diaphysis. The carpus, the proximal and distal extremities of the metacarpi and the second phalanges of all digits had no cartilaginous templates **fig (15)**.

The bones of the hind limb (os coaxe, femur and tibia and fibula) of the **control group** were grossly longer than those of the treated fetuses with AFB. The os coaxe of the **treated group** had a very small ossified pubis than that of the control one fig (16A &B). The distal extremity of tibia, central tarsal bone as well as the distal raw of tarsus in the **control group** had cartilaginous templates and the talus and calcaneus showed ossification. While, the **treated group** showed no cartilaginous templates for the distal extremity of tibia, central and distal raws of tarsaus and showed the commencement of ossification of talus and calcaneus fig (17A, 17B &18)

Discussion

An animal experiment is the first scientific preference to learn whether a substance does have a teratogenic effect or not. It is the most acceptable way for the drug producers to determine the potential teratogenic effects of any given drug on the human health. In relation to that, this study documented the teratogenic effects of AFB1 in rabbits which have similarity to human in the early developmental pattern (Beaudion et al, 2003) and the extra-embryonic membranes were more closely resemble to that of human (Foote and Carney, 2000).

On this experimental study, AFB1 was administered to rabbits orally (through gastric probe) which was appear to be the most accepted and accurate way to give a fixed dose (Arora, 1982).

Doses of AFB1 administration in pregnant rabbits was applied in various studies as (1 and 3ppm)/ kg added to diet from day zero of conception to one weak post-partum (Reddy and Rao, 2001). While, Wangikara, et al. (2005) used different doses 0.025, 0.05 and 0.1 mg/kg by gastric intubation during the whole length of organogenesis period and inferred that 0.1mg/kg body weight was the minimum teratogenic dose. In our study, an oral dose of 0.05mg/kg was applied to pregnant.

Time of AFB1 intoxication in pregnant rabbits in the current work, from 6^{th}-18^{th} days of gestation which is recognized as the period of organogenesis in rabbits as reported by Petrere et al, (1993). In this concern, Stanley and Bower (1986) and Vickers and Brackley, (2002) clarified that development of embryo is affected by teratogens mostly during the process of organogenesis

In our study, there were no mortalities of any fetuses of any intoxicated dams during the period of experiment a result which in a line with Wangikara, et al., (2005) for the same dose, toxin, period of intoxication and animal.

The mean fetal weights and crown rump lengths of intoxicated fetuses were significantly decreased than the control ones, a result agreed with Wangikara, et al. (2005) for the same toxin and animal species intoxicated with 0.1mg/kg during the same period. The latter authors added that the dose of 0.05mg /kg AFB1 causes non-significant decease for these parameters.

The findings of Wangikara, et al. (2005) for micro-ophthalmic eyes, enlarged eye socket and wrinkled skin for fetuses for intoxicated dams with a dose of 0.1mg/kg AFB1, also noticed in the present work in addition to sever subcutaneous congestion at different regions including abdomen and head.

The fusion of auriculo ventricular valves and narrow ventricular lumen for fetuses of intoxicated dams with a dose of 0.1mg/kg AFB1 were observed by Wangikara, et al (2005).

Our work revealed small sized heart with shallow inter-ventricular grooves and wider ventricular lumen in the same animal intoxicated by a dose of 0.05mg/kg AFB1.

Regarding the histopathological findings, the hepatocyte suffered from vaccuolation, distortion of normal trabecular structure, some hepatocytes had pyknotic nucleus and extra medullary hematopoiesis was subnormal, the findings which seen by Wangikar et al (2005) in treated rabbit fetuses with a dose of 0.1mg/kg AFB1. The last authors recorded that the feti of treated rabbits with a dose of 0.05mg/kg AFB1 showed milder degrees even comparable to the control.

Concerning kidney lesions, granular vacuolated cytoplasm and prominent casts of some renal tubules and degeneration as well as atrophy of the glomeruli were observed. These lesions also confirmed by Wangikar et al, (2005) in the same animal with the same dose of toxin and with AFB1 + OTA of 0.1mg+0.1mg respectively in rabbits fetuses and El-Tahan (2013) in treated rat fetuses with a dose of 0.1mg/kg AFB1.

The dermis of the eye lids had few number of the hair follicle in the fetuses under investigation, while Wangikar, et al. (2005) found mild lenticular degeneration in fetuses treated with both doses of 0.05mg and 0.1mg /kg AFB1 in the same animal.

However, the information on detailed skeletal anomalies due to AFB1 in rabbit fetuses is not available; the present study is not passible to make a detailed discussion. Where Wangikar, et al., (2005) mentioned only there was incomplete ossification of the skull bones of fetuses intoxicated with a dose of 0.1mg/kg AFB1. In this concern, El-Tahan. (2013) reported incomplete ossification of the skull in intoxicated rat fetuses with the same toxin and a dose of 0.1mg/kg.

References

Argyrous, G., (2005): *Statistics for Research with a guide to SPSS*, 2, Sage Publications, London

Arora, R.G.; Frolen, H and **Nilson, A.**, **(1981):** Interference of mycotoxins with prenatal development of the mouse. I.Influence of aflatoxinB1, ochratoxin A and zearalenone. Acta. Vet. Scand. 22: 524–533**.**

Bancroft, J and **Gamble, M., (2007):** Theory and practice of histological Techniques. 6th ed.

Beaudion, S.; Barbet, P and **Bargy, F., (2003):** Developmental Stages in the Rabbit Embryo: Guidelines to Choose an Appropriate Experimental Model. Fetal. Diagn. Ther.18:422-427.

Brendel, R; Duhamel, K.C and Shepard, T.H., (1989): Embryotoxic drugs. Biol Res. Preg, 6(1):1–54.

Butler, W.H and **Wigglesworth, H.S.**, **(1966):**The effects of aflatoxin B1 on the pregnant rat. British J. Exp. Pathol. 47: 242–247.

Clark, J.D.; Jain, A.V.; Hatch, R.C and **Mahaffey, A., (1980):** Experimentally induced chronic aflatoxicosis in rabbits. Am. J. vet. Res, 41:184-1845

Diekman, M.A and **Green, M.L,** (1992): Mycotoxins and reproduction in domestic livestock. Animal Science, 70: 1615-1627.

Dipaolo, J.A.; Ellis, J and **Erwin, H.E.**, **(1967):** Teratogenic response by hamsters, rats and mice to aflatoxin. Nature 215: 638–669.

El-Tahan, A.S., (2013): A study on the effects of mycotoxins on pregnant rats. Master thesis, faculty of veterinary medicine, Suez Canal University, pathology department.

Foote, R.H and **Carney, E.W., (2000):** The rabbit as a model for reproductive and developmental toxicity studies. Reprod. Toxicol.14: 477–493.

Hussein, H.S and **Brasel, J.M**., **(2001):** Toxicity, metabolism and impact of mycotoxins on human and animals. Toxicology. 167:101–134.

Inouye, M., **(1976):** Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S.Cong. Anom, 16:171–3.

Mayura, K.; Abdel-Wahhab, M.A.; McKeinzie, K.S.; Sarr, A.B.; Edwards, J.F.; Naquib, K and **Phillips, T.D., (1998):** Prevention of maternal and developmental toxicity in rats via dietary inclusion of common aflatoxin sorbents: potential for hidden risks. Toxicol. Sci. 41: 175–182.

Nomina Anatomica Veterinaria., (2005): 5th Ed. Prepared by International Committee on Veterinary Gross Anatomical Nomenclature (I. C.V.G.A.N) and authorized by the General Assembly of the World Association of Veterinary Anatomists (W. A. V.A), Konxiville, TN, USA.

Nomina Embryologica Veterinaria., (2003): 2nd Edition. International Committee on Veterinary Gross Embryological Nomenclature (I. C.V.E.N) and authorized by general assembly of the world association of Veterinary Anatomists (W.A.V.A) Konxiville, TN, USA.

Petrere, J.A.; Rohn, W.R.; Grantham.; L.E and **Anderson, J.A., (1993):** Food restriction during organogenesis in rabbits: effects on reproduction and the offspring. Fund. Appl. Toxicol, 21:517–522.

Reddy, G and **Rao, D.G.K., (2001):** Teratological effects of aflatoxin in rabbits. Ind. Vet. J, 78: 900–902.

Schmidt, R.E and **Panciera, R.J, (1980):** Effects of aflatoxin on pregnant hamsters and hamster fetuses. J. Comp. Pathol, 90: 339–347.

Sharma, A and **Sahai, R., (1987):** Teratological effects of aflatoxin on rats (Rattus novegocus). Ind. J. Anim. Res. 21 (1):35–40**.**

Stanley,F.C and **Bower,C., (1986):** Teratogenic drugs in pregnancy. Med J Australia, 11–12(145): 596–9.

Trail, F.; Mahanthi, N and **Linz, J., (1995):**Molecular biology of aflatoxin biosynthesis. Microbiol, 141 (4): 755 –765.

Vickers,M and **Brackley,k., (2002):** Drugs in pregnancy. Curr. Obstet. Gynaecol, 12:131–7.

Wangikar, P.B.; Dwivedi, P and **Sinha, N., (2004b):** Effects in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. I. Maternal toxicity and fetal anomalies. Birth Defects Research Part B: Developmental and Reproductive Toxicity, 71 (6): 343–351.614.

Wangikar,P.B.;Dwivedi, P.; Sharma, A.K and **Sinha, N., (2005):** Effects of aflatoxin B1on embryo fetal development in rabbits. Food and Chemical Toxicology, 43:607–615.

World Health Organization, International Agency for Research on Cancer (IARC), (1993): Monographs on evaluation of carcinogenic risks to humans.

Young, A.D.; Phipps, D.E and **Astroff, A.B., (2000):**Large-scale double staining of rat fetal skeletons using. Alizarin red-S and Alcian blue. Teratology 61:273–6.

Table (1): Mean ±SD for fetuses at 29th day of gestation of both control and treated with (0.05mg/kg AFB1)
P- Value = .0001.
Within the same raw means with different superscripts considered highly significant as ($p \leq 0.01$).

Measurements	Control fetuses	Treated Fetuses	T	P-value	Df
Body weight (gm.)	54.28^{a}±8.26	28.00^{b}± 1.29	8	0.001	12
CRL (cm)	11.57^{a}±0.47	9.84^{b}±0.21	9	0.001	12
Liver and gallbladder weight (gm.)	3.23^{a}±0.23	2.52^{b}±0.296	5	0.001	12
tomach and intestine Weight (gm.)	2.47^{a}±0.11	1.84^{b}±0.11	10	0.001	12
Heart and lungs Weight (gm.)	1.89^{a}±0.092	1.69^{b}±0.11	4	0.002	12
Kidneys Weight (gm.)	0.33^{a}±0.0365	0.247^{b}±0.011		0.001	12

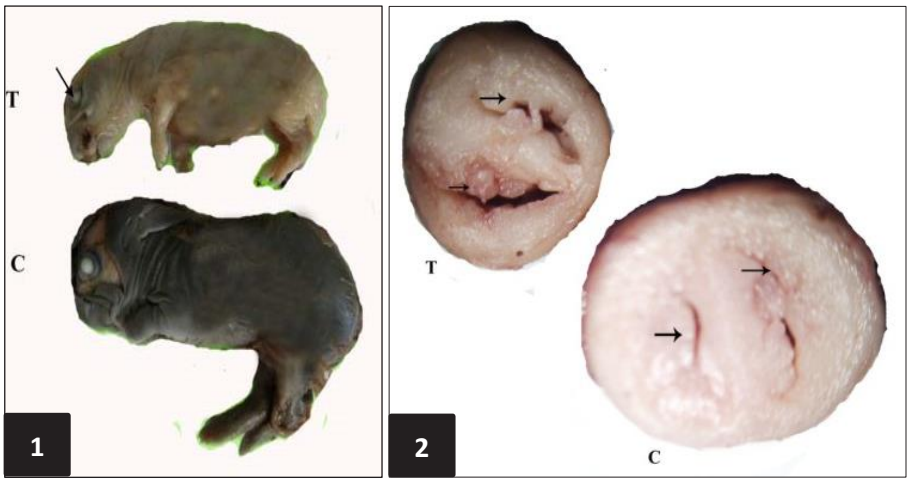


Fig (1): A photograph showing significant decrease of CRL of the treated (**T**) fetuses than the control (**C**) with micro-ophthalmic eye (**arrow)**

Fig (2): A photograph showing cross section of ventricular mass of treated fetus (**T**) having wider ventricular lumen eye (**arrow)** than that of control (**C**).

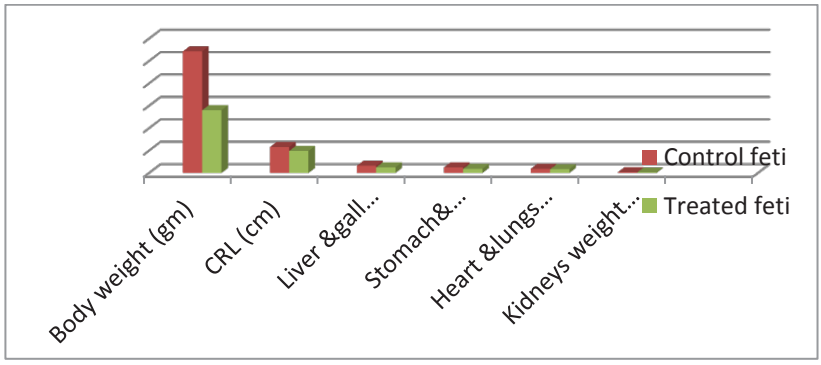
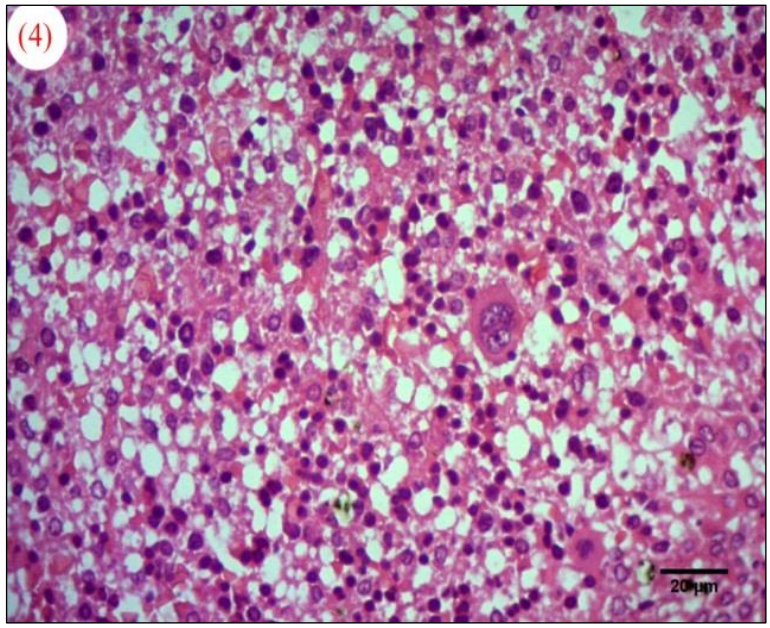


Fig (3): A histogram showing gross parameters for both control and treated fetuses



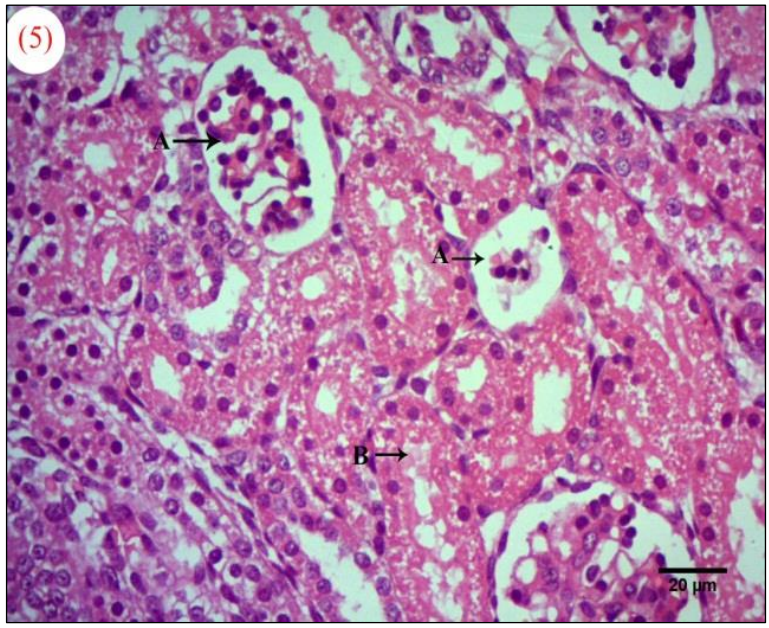


Fig (4): A photomicrograph showing prominent vaccuolation of the hepatocytes and distortion of hepatic cords of treated fetus liver. H&E 400X.

Fig (5): A photomicrograph showing vacuolated granular cytoplasm of the renal tubules, atrophy of glomeruli (**A**) and prominent casts (**B**) of treated fetus. H&E 400X.

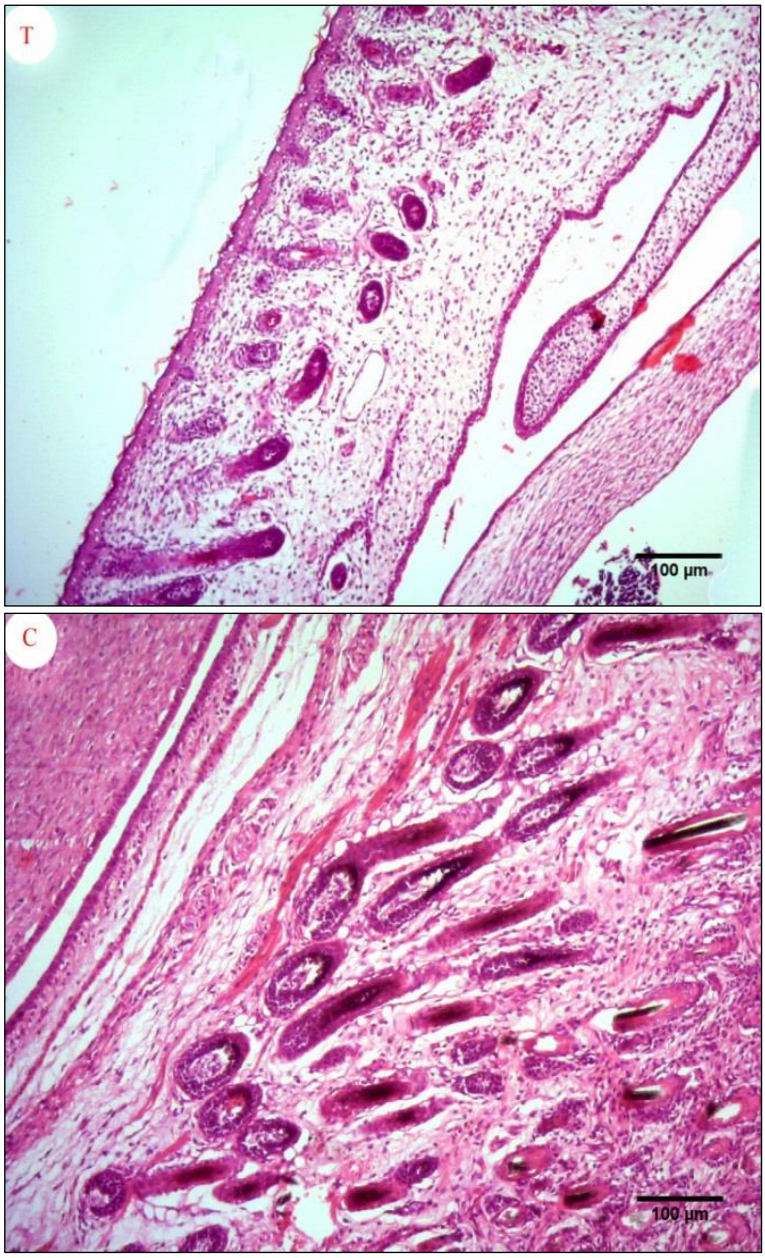
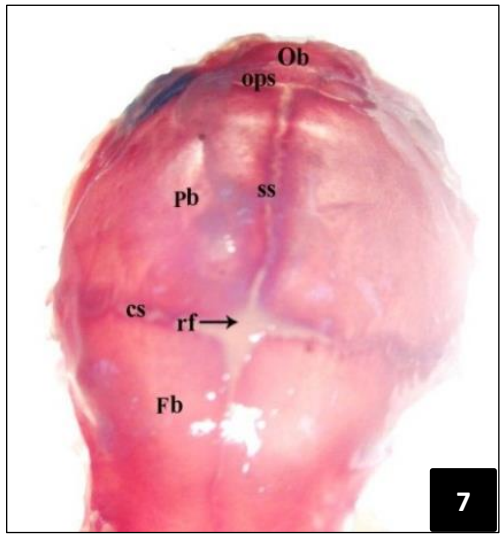


Fig (6): A photomicrograph showing lower number of hair follicles within eye lids skin of treated fetus (**T**)with a dose of 0.05mg/kg compared to control (**C**) at 29th day of gestation H&E 100X



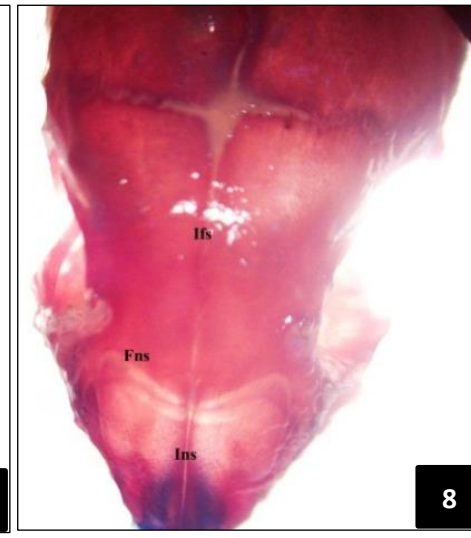


Fig (7): A photograph of dorsal view of skeleton craniale of control fetus showing open small rostral fontanelle (**rf**) and well established sutures (occipito-parietal (**OPS**), sagittal suture (**SS**) and coronal suture (**CS**). Parietal bone (**Pb**) and frontal bone (**Fb**).

Fig (8): showing well established and closed interfrontal (**Ifs**), frontonasl (**Fn**) and internasal (**Ins**) sutures

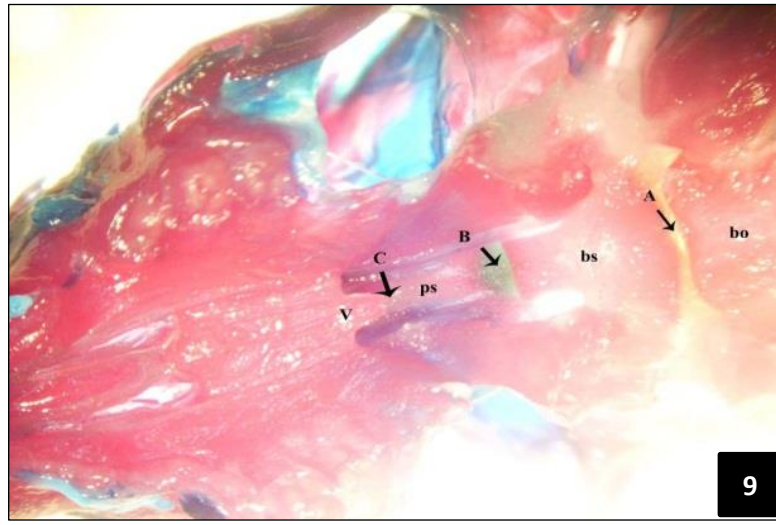


Fig (9): A photograph of ventral view of skeleton craniale of control fetus showing nearly complete ossification (**A**) between basioccipital (**bo**) and basisphenoid (**bs**) bones as well as between the pre sphenoid (**ps**) and vomer (**V**) bones (**C**).while the basisphenoid and pre sphenoid bones were still connected by a plate of cartilage (**B**).

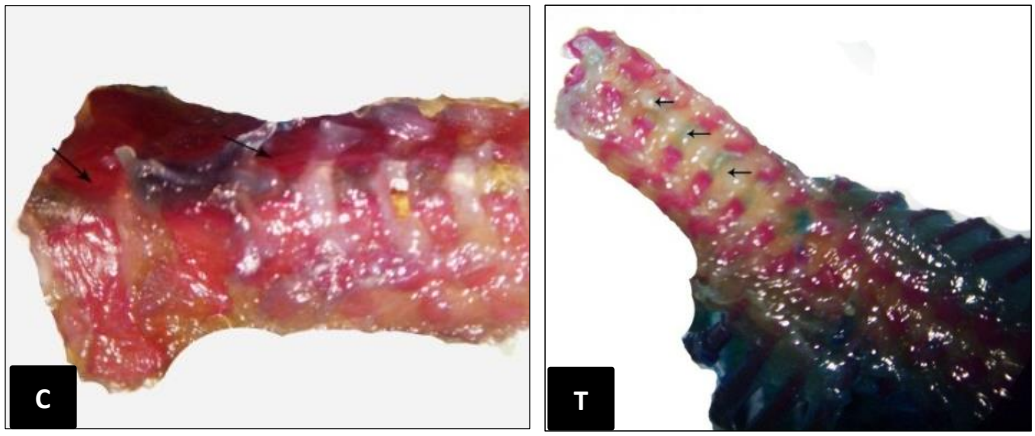


Fig (10): Photographs of cervical vertebrae of control fetus (C) with complete ossification of their arches and treated (**T**) with cartilaginous (laminae) (**arrows**).

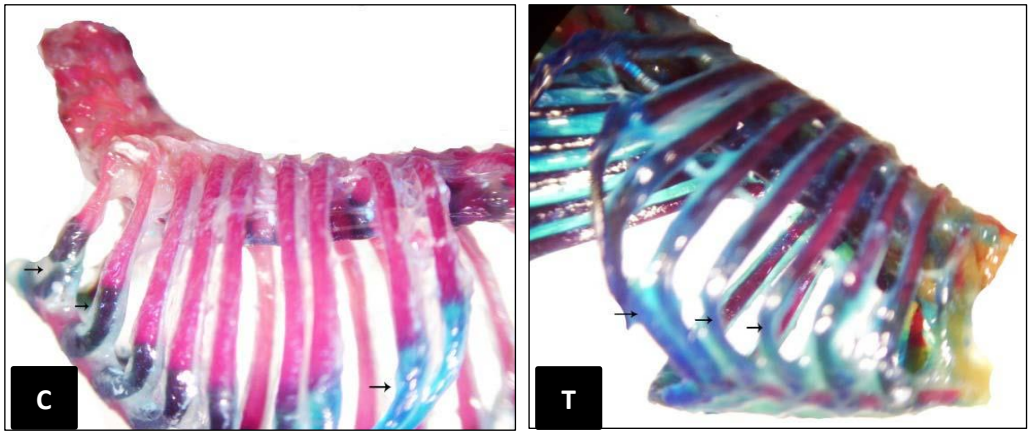
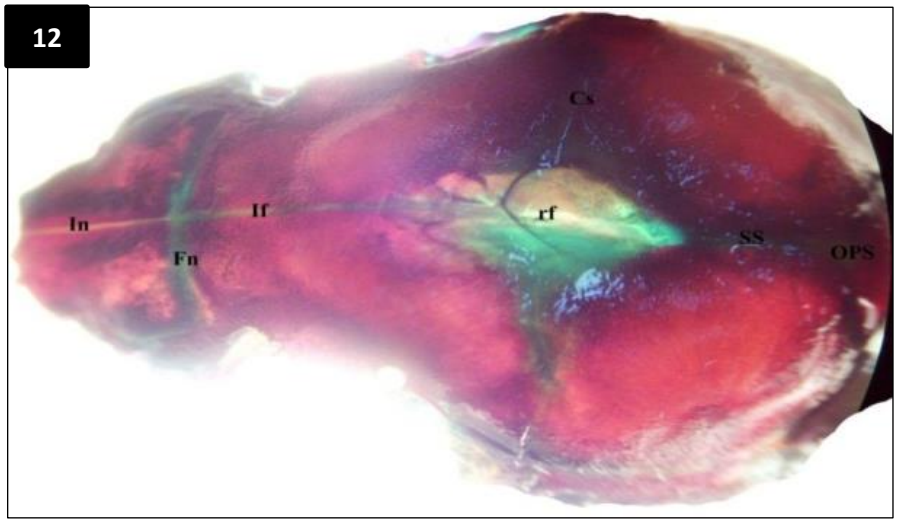


Fig (11): A photograph of control fetus (**C**) showing complete ossification of ribs and treated fetus (**T**) with a dose of 0.05mg/kg AFB1 showing incomplete ossification of ribs (**arrows**)



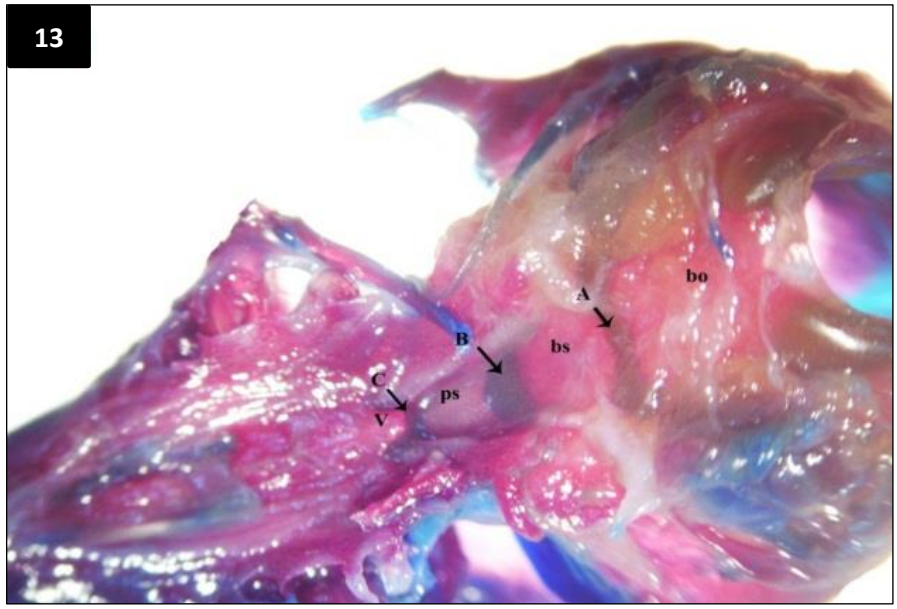


Fig (12): A photograph of skeleton craniale of treated fetus showing wide rostral fontanelle (**rf**), well established smooth edged sutures (sagittal suture (**SS**), coronal suture (**Cs**), occipito-parietal suture (**Ops**), internasal suture (**In**), frontonasal suture (**Fn**) and interfrontal suture (**If**).

Fig (13): A photograph of ventral view of skeleton craniale of treated fetus, showing cartilaginous plates (**A**) between pars basilaries occipitale (**bo**) and pars basisphenoidale bones (**bs**), (**C**) between the os pre sphenoidale (**ps**) and vomer (**V**), as well as (**B**) between the os basisphenoidale and os pre sphenoidd bones.

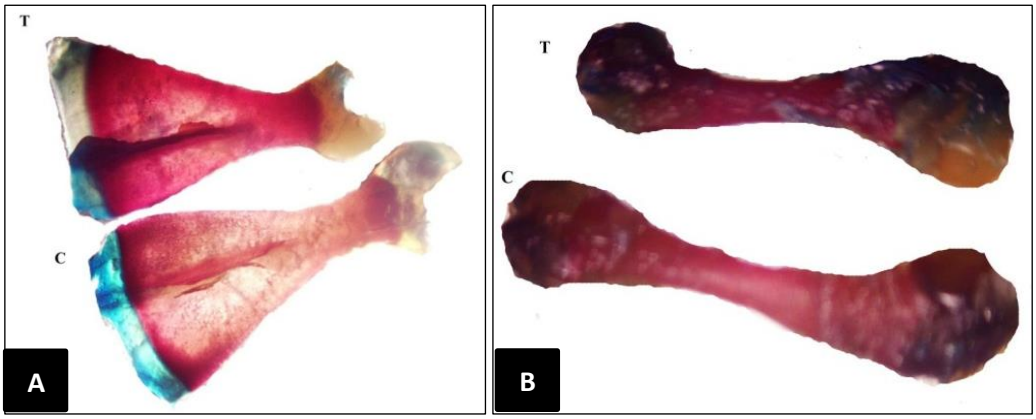


Fig (14A): A photograph showing scapula of control fetuses (**C**) was grossly longer than that of the treated fetuses (**T**).

Fig (14B): A photograph showing humerus of control fetuses (**C**) was grossly longer than that of the treated (**T**) fetuses.

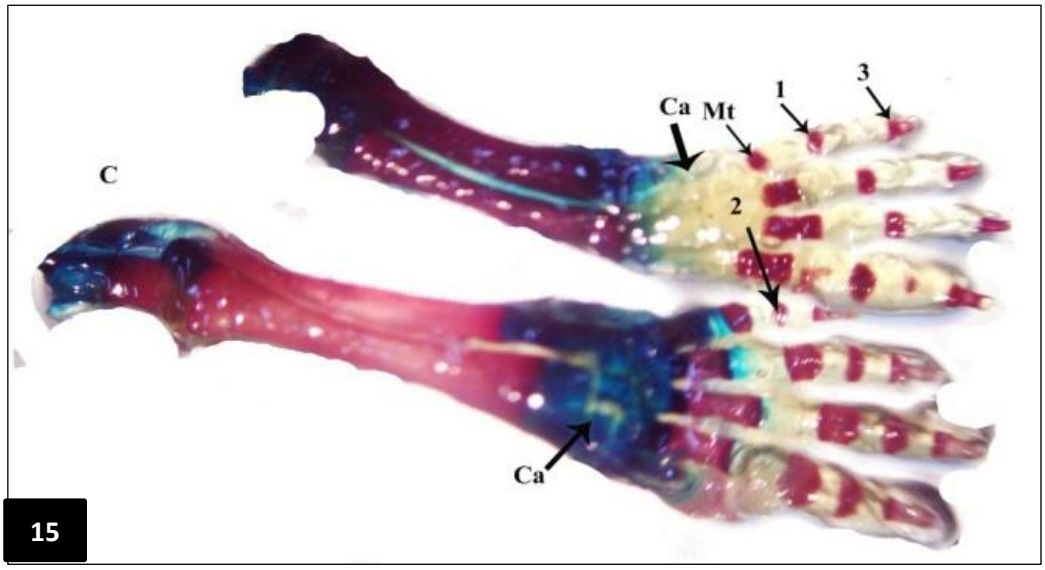


Fig (15): A photograph showing grossly longer radius and ulna of control fetuses (**C**) than that of the treated fetuses (**T**) also there were no cartilaginous templates of, carpus (**Ca**), proximal and distal extremity of metacarpi (**Mt**) and 2nd phalanx of the treated fetuses. 1st phalanx (**1**), 2nd phalanx (**2**) and 3rd phalanx (**3**).

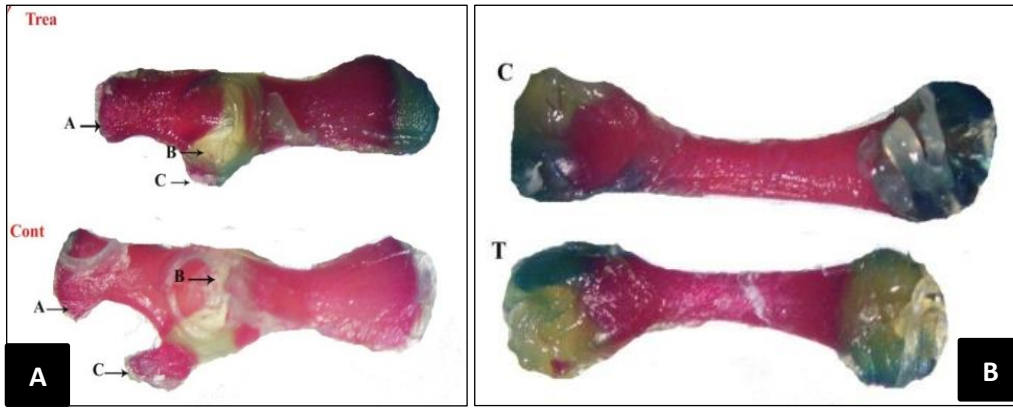


Fig (16A): A photograph showing os coaxe of control fetuses (**Cont**) was grossly longer than that of the treated (**T**) ones also, the os pubis of treated fetuses represented as a small ossified bone (**C**).

Fig (16B): A photograph showing os femur of control fetuses (**C**) was grossly longer than that of the treated ones (**T**).

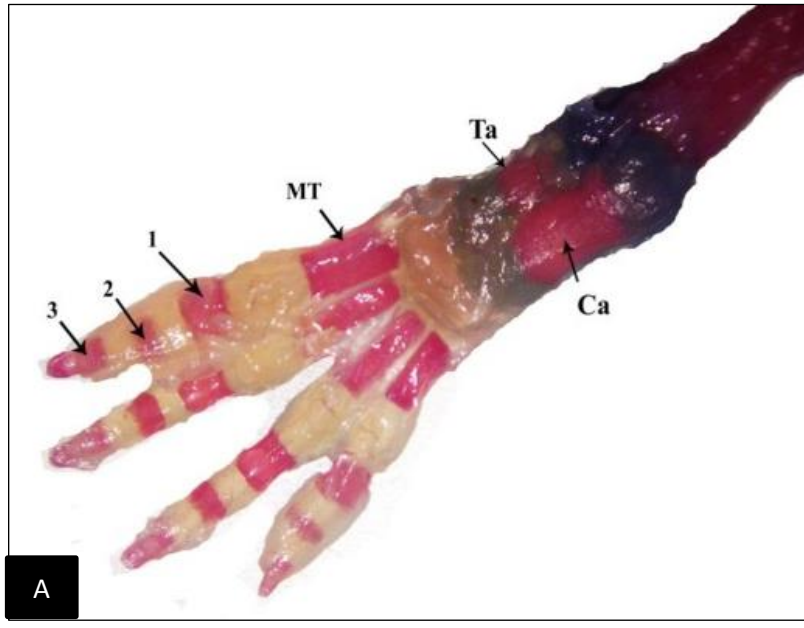


Fig (17A): A photograph showing completely ossified metatarsus (**MT**) and digital phalanges (**1, 2 &3**) of the control fetuses, as well as tarsal bones the talus (**Ta**) and calcaneus (**Ca**) were the only ossified and the remaining bones of the tarsus had a cartilaginous templates.

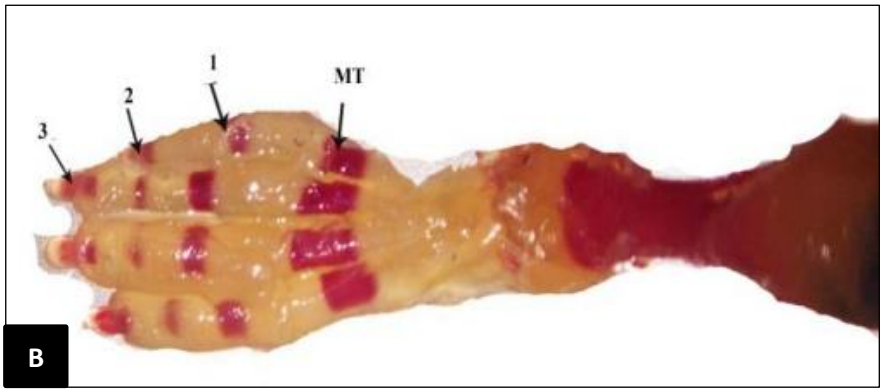


Fig (17B): A photograph showing metatarsus (**MT**), tarsus with beginning of ossification of talus and calcaneus while the rest had no cartilaginous templates and ossified digital phalanges (**1, 2 &3**) of the treated fetuses with 0.05mg /kg AFB1 at 29th days of gestation.

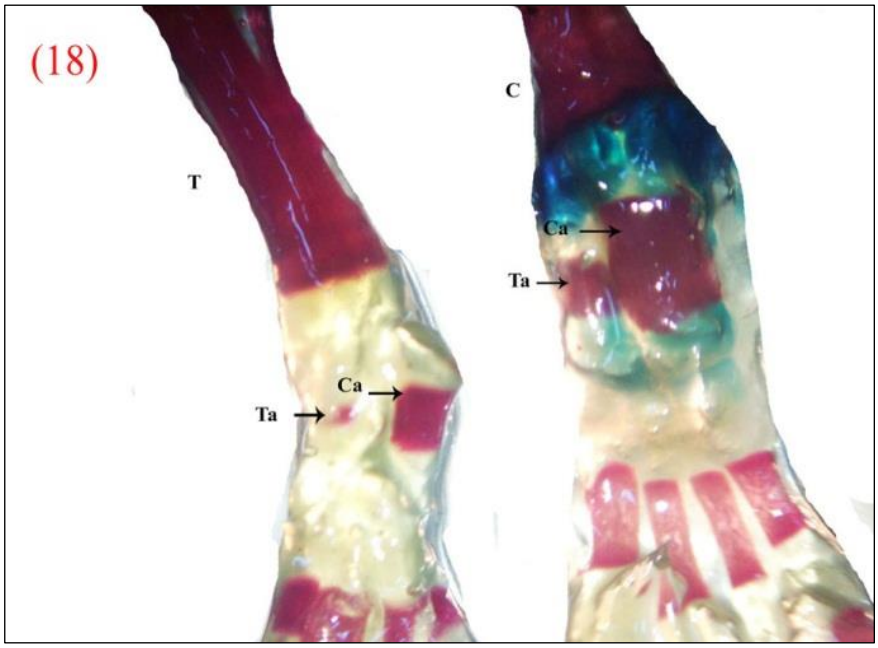


Fig (18): A photograph of planter aspect of pes region of control fetuses (**C**) and treated one (**T**) showing tarsus with beginning of ossification of talus (**Ta**) and calcaneus (**Ca**) while the rest had no cartilaginous templates in the treated one