

EXPECTED AMELIORATIVE EFFECT OF FENNEL OIL IN THE TERATOGENICITY OF DEXAMETHASONE TREATED ALBINO RATS

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ABSTRACT

Dexamethasone, a corticosteroid, is analogous to a natural hormone produced by adrenal glands. It is employed to exchange this chemical when your body doesn't make enough of it. It relieves inflammation (swelling, heat, redness, and pain) and is employed to treat certain sorts of arthritis; skin, blood, kidney, eye, thyroid, and intestinal disorders (e.g., colitis); severe allergies; and asthma. Dexamethasone is treating certain sorts of cancer. There are studies tells us that administration of dexamethasone to pregnant women increases the risk of birth defect in the offspring and possible also other adverse effect in the exposed infant. The present work studied the ameliorative effect of fennel oil against teratogenic effect induced by the dexamethasone in albino rats. The animals were divided into 4 groups: group 1 (control), group 2: the pregnant rat orally given fennel oil (one ml/kg body weight), group 3: the pregnant rat orally-administered with human equivalent dose 0.13 mg/Kg dexamethasone and group 4: the pregnant rat oral administered with dexamethasone followed by fennel oil from 5th day of gestation to 19th day of gestation. The pregnant rats were sacrificed under anesthesia at 20th day of gestation. The uteri horns were isolated, and the numbers of resorbed sites were determined. In addition, the morphological examination and skeletal staining of fetuses and the histological studies on fetal liver have been done. The Result showed decrease in both corrected body weight and placenta weight of pregnant mother and high incidence of resorption. Many hematomas in many parts of body and other malformations represented in deformed fore limb were recorded in group treated with dexamethasone. The skeletal malformation summarized as less degree of ossification, complete unossified bones, wavy ribs, curved ribs and costal separation in group treated with dexamethasone. The histopathological change summarized in pyknoses of some liver cells, necrotic area and fatty degeneration were noticed in histology of liver in group treated with dexamethasone. While the group treated with dexamethasone followed by fennel oil showed ameliorative effect in which the number of resorbed sites and hematoma were decreased, more bones were completely ossified and there are not any ribs anomalies were recorded. There was improvement in hepatic tissue as the architecture become more organized. Therefore, the fennel oil is given with dexamethasone to avoid embryotoxic effect induced by dexamethasone during pregnancy.

KEYWORDS

Dexamethasone, Fennel oil, Wistar rat, Pregnant rats, Histopathology, Embryotoxicity

INTRODUCTION

Teratology is the study of examining the imperfections that happen at the birth or at various phases of pregnancy. During exposure to a dangerous specialist, a creating developing life will show diverse level of reaction (death or abnormality) (Wise, 2016).

Dexamethasone, (11 β , 16 α)-9-Fluoro-11, 17, 21-trihydroxy-16-methylpregna-1, 4diene-3, 20-dione. MK-125 or dexamethasone is a corticosteroid fluorinated at position 9 used to treat rheumatic, endocrine, dermatologic, collagen, allergic, ophthalmic, respiratory, gastrointestinal, neoplastic, hematologic, edematous, and other conditions (Cavanagh and singh, 1970) It is structurally similar to other corticosteroids like [hydrocortisone](#) and [prednisolone](#) (Bunim *et al.*, 1958).

Dexamethasone is one of the glucocorticoid drugs that has many serious adverse effects when used in large doses or long-term ([Harris *et al.*, 2015](#)).

Dexamethasone had previously been categorized as a Pregnancy Category C drug, which means that data from animal studies mentioned potential fetal hazards, but no sufficient data from human clinical trials or studies were available at the time of approval.

Foeniculum vulgare known as saunf or fennel. It is biennial medicinal plant belongs to family named Apoaceae (Umbelliferae). Also, *Foeniculum Vulgare* known in Indian, Chinese, Greek and Egyptian civilization for medicinal aims and purposes and human uses (Aboelsaoud, 2010). Fennel is a herb medicinal plant which has many properties made it protective against many diseases. And it's known in Egyptian traditional medicine for many diseases (Hanan *et al.*, 2013). Composition of Fennel seeds have many different nutrients like sugars (Cataldi *et al.*, 1998), minerals (Özcan *et al.*, 2008), essential fatty acids (Vardavas *et al.*, 2006), The major constituents of essential oil are anethole, estragole and fenchone (Mimica-Dukic' *et al.*, 2003). Also, fennel seeds have vital vitamins, fibers and protein and rich with flavonoids and essential oil (Das *et al.*, 2013). These all compounds and their nutritional values. Fennel oil has properties as antioxidant (Diaz-Maroto *et al.*, 2005), Hepato-protective (Ozbek *et al.*, 2003).

The objective of this study is to inquiry the expected ameliorative effect of fennel oil against the embryotoxicity induced by dexamethasone administration.

MATERIALS AND METHODS

Animals and housing conditions:

All the experimental protocols and procedures used were verified by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/Comp & Emb /11/20).

In teratological studies the rat is preferred since it has a short duration of pregnancy (about 21 days), a high fertility rate, genetic stability and a very low rate of spontaneous malformation (about 0.1 percent) (Tuchmann-Duplessis, 1966 and 1977; Wilson, 1973; Banerjee and Durlou, 1973).

Healthful adult male and female rats (*Rattus norvegicus*) of weight 170-180 grams were used in the present investigation. The animals have been taken from the animal house of the Faculty of Veterinary, Cairo university- Egypt. Animals could acclimatize, also it was caged separately, males in cages and females in others. For one week before beginning the study, all rats be kept in a quite non stressful environment. The rats were fed with water and normal, standard food pellet libitum. All the operational guidelines in the routine husbandry, housing, handling, and experimental procedures were approved by the committee for animal ethics and experiments. Female rats were mated in the proportion of two females for every male overnight. Each morning a vaginal smear was taken using saline to check for the presence of sperms or plug in the vagina and placed on microscope slides using magnification of 100 x with a drop of methylene blue solution. the presence of sperms in the vaginal smear is the zero day of gestation (Mcclain and Becker, 1975).

Experimental procedure:

Female rats were administrated orally (by gavage) once daily in the morning from 5th day where the organogenesis phase was begun to 19th day of gestation.

Drug

- Dexamethasone the recommended maximum dose for human is 16.6 mg/kg. The dose modified to suit the weight of rats (0.13 mg/kg) according to (Shannon et al., 2008). One tablet of 0.73 mg dissolved in 10 ml distilled water. Each rat given 0.1 ml of the prepared solution. The rats were administered orally.□

□

- Fennel oil (1ml/kg) was purchased from the supermarket.□

Experimental Design

Time of administration: Scheduled from the 5th to 19th day of gestation.

Experimental groups

- • Group (A): Control group (6 rats) received distilled water.□
- • Group (B): The pregnant rats of this group (6 rats) were orally given one ml/kg fennel (Rabeh *et al.*, 2014).□
- • Group (C): The pregnant rats in this group (6 rats) given dexamethasone via gastric tube.□
- • Group (D): The pregnant rats in this group (6 rats) received dexamethasone drug followed by the fennel oil.□ **Maternal-Fetal Investigation**

On the 20th day of gestation, the weight of all pregnant rats of group (A-D) were recorded to calculate the mother corrected body weight gain then all rats were anesthetized by using the sodium pentobarbital 60 mg/kg then sacrificed by decapitation, The uteri horns were separated and the weight of uterus was recorded also the number of live fetuses, dead fetuses, resorbed sites were determined, The weight of all placenta were recorded and checked carefully, the checking for any external anomalies was performed under a dissecting microscope. The post-implantation loss index (Number of implantation sites - Number of live fetuses)/ Number of implantation sites x100 was calculated.

Skeletal examination

The fetuses were examined to determine any skeletal anomalies by using skeletal staining technique. The fetuses were skinned and eviscerated after they were fixed in 95% ethyl alcohol for 10 days and placed into pure acetone soln. to remove the fats. The fetuses were stained in alcian blue and alizarin red stains: Alcian blue: 0.3 g in 100ml 70% ethanol (one volume alcian blue). Alizarin red: 0.1 g in 100 ml 95% ethanol (one volume alizarin red). volume glacial acetic acid (100 ml). 17 volumes of 70% ethyl alcohol (1700 ml). Then the fetuses were stained for four days at 40°C. The specimens were washed carefully for two hours in tab water. To allow the skeleton become clearly visible through the surrounding tissue, the fetuses were transferred into an aqueous soln. of 2% KOH (two g KOH in 100m distilled water) for three days, then the fetuses transferred into aqueous solution of 20% glycerin containing 1% KOH until the skeleton become clearly visible. Into 50%, 80% and 100% glycerin solution, cleared fetuses were placed successively for seven days each step. Then the cartilage part will be appeared blue while the bone will be appeared red. Finally, the stained skeletons were examined by using the dissecting binocular microscope to determine skeletal malformation such as, degree of the bone ossification, shape and the number of different bone parts (Young and Heath, 2000).

Histological Examination

Cross-sections through the fetal liver at 20th day of gestation were dissected out and the excess of the fats were removed and then were immediately fixed in 10% buffered formalin for histological examination for 24 hours and were processed for paraffin sectioning by dehydration in different concentrations of alcohol (methyl, ethyl and absolute ethyl), cleared with xylol and embedded in paraffin blocks. Sections of about cut 5cm thick, collected on glass slides, deparaffinized, stained with hematoxylin and eosin and. By using light microscope all sections were examined and photographed. All methods were applied according to Drury and Wallington (Drury and Wallington, 1980; Khidr *et al.*, 2017).

Statistical analysis

Data will be presented as mean \pm standard error mean in order to determine differences between groups, analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc analysis was used for multiple comparisons

between different groups. The level of statistical significance is sitting at probability $P < 0.05$.

ETHICAL CONSIDERATIONS

All the experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/Comp & Emb /11/20).

RESULTS

Effect on mothers

The corrected body weight of control and fennel oil groups were (47.95 \pm 2.81) & (13.65 \pm 6.82) respectively. While the corrected body weight of pregnant mother treated orally with dexamethasone from 5th to 19th day of gestation was decreased to (-43.6 \pm 4.90) with significant difference as compared with control and fennel oil groups. On the other hand, the corrected body weight of Pregnant rats treated orally with dexamethasone & 1ml/kg fennel oil) group was increased to (-5.70 \pm 1.95) with significant difference as compared with dexamethasone group (Table 1 & Fig. 1).

The average pre implantation loss index and the average post implantation loss index of pregnant rats treated with dexamethasone was increased to (36.20 \pm 8.21), (18.53 \pm 2.28) respectively with significant difference as compared with control and fennel oil groups and the average number of resorbed site was increased to

(1.66 ± 0.21) with significant difference as compared with control and fennel oil groups (Table 2 & Fig. 7,8). The average pre implantation loss index and post implantation loss index of pregnant rats treated with (0.13 mg/kg dexamethasone, 1ml/kg fennel oil) was decreased to (16.37 ± 2.29) & (6.66 ± 4.21) respectively and the average number of resorbed site was decreased to (0.66 ± 0.42) with significant difference as compared with dexamethasone group (Table 1 & Fig. 1).

Effect on fetuses

The average number of viable fetus of pregnant rats treated with 1ml/kg fennel oil was (9.0 ± 1.34). The average number of viable fetus of pregnant rats treated with (dexamethasone) from 5th to 19th day of gestation was decreased to (7.33 ± 0.557) with non-significant difference as compared with control and fennel oil groups. While the average number of viable fetus of pregnant rats treated with (dexamethasone & 1ml/kg fennel oil) was increased to (8.0 ± 0.00) with non-significant difference as compared with dexamethasone group (Table 1 & Fig. 1). In addition, the average fetus weight of control group was (3.73 ± 0.057) and that of pregnant rats treated with 1ml/kg fennel oil was (2.86 ± 0.033). The average fetus weight of pregnant rats treated with (dexamethasone) from 5th to 19th day of gestation was decreased to (2.40 ± 0.07) with significant difference as compared with control and fennel oil groups. While the average fetus weight of pregnant rats treated with (dexamethasone & 1ml/kg fennel oil) group was increased to (3.17 ± 0.06) with significant difference as compared with dexamethasone group (Table 1 & Fig. 1).

The fetus from control and fennel oil groups revealed normal shape, correct body weight and length and appeared with straight dorsal side, the eyes are closed, with lower and upper eyelids and have well developed ear pinnae. Both fore and hind limbs are constituted from well-developed bones and they had well-formed digits at their extremities. The abdominal region acquires a cylindrical shape which ends with the tail (Fig. 2). The average number of hematoma in control group and group treated with 1ml/kg fennel oil was (0.333 ± 0.333) which mean it is at the normal range. The average number of hematoma of fetuses of pregnant rats treated with

(dexamethasone) from 5th to 19th day of gestation was increased to (2.66 ± 0.55) with significant difference as compared with control and fennel oil groups. The average number of hematoma in group treated with (dexamethasone, 1ml/kg fennel oil) group was decreased to (2.0 ± 0.63) with significant difference as compared with dexamethasone group. Also, there are malformations represent as deformed fore limb in a group treated with dexamethasone (Fig. 3).

While the fetuses of pregnant rats treated with (dexamethasone and 1ml/Kg fennel oil) showed improvement in morphology with no abnormalities were detected (Fig.4).

Skeletal anomalies:

In control group and group treated with fennel oil Showed completely ossified bones of skull, ribs, sternum, vertebral columns, pectoral girdle and fore limb, pelvic girdle and hind limb stained red (Fig. 5).

There were signs of skeletal anomalies in fetus of pregnant rats treated orally with dexamethasone which represented incomplete ossification of frontal, nasal, mandible and bones as parietal (Fig. 6). Also, the sternum showed some anomalies as incomplete ossified sternbrae (Fig. 7) and the ribs anomalies represented in the wavy ribs, curved ribs and costal separation, in contrast, there are not any ribs anomalies were recorded (Fig. 8 & Table 2).

Also, there was a noticeable improvement in group treated with dexamethasone and fennel oil which represented in showing completely ossified bones of Maxilla, mandible, parietal, frontal, in addition complete ossified bones of ribs and vertebral column, Pelvic girdle and Hind limb, Pectoral girdle and Forelimb and the sternum (Fig. 9).

The histological study of fetal liver

Fetal liver histology is different from adult liver, the fetal liver at 19th day of pregnancy is covered with a very thin capsule consisting of a single elongated mesothelial cell.

1) Control group:

Normal structure of the liver tissue observed. The central vein with its intact endothelial lining was normal, normal hepatocytes, blood sinusoids and numerous of erythroblasts were all in normal shape (Fig. 30).

2) Oil group:

Showing normal construction of the liver tissue. Normally The central vein, normal Hepatocytes, plentiful erythroblasts and blood sinusoids. In oil group we observed that there are no changes in comparison with control group (Fig. 31).

3) Dexamethasone group:

We observed that there is loss normal architecture of hepatic tissue, and we found that there are empty areas are scattered all over the tissue, rupture of epithelial cells lining central vein, hemorrhage and multiple number of erythroblasts can be seen. Degenerated hepatocytes, karyolysis nuclei and hemosiderin pigment have been seen (Fig. 32).

4) Dexamethasone and oil group:

We observed good appearance of the hepatic tissue. Normal central vein, normal hepatocytes, normal blood sinusoids and numerous of erythroblasts were normal. Fennel oil improved the histopathological changes induced by dexamethasone intake (Fig. 33).

DISCUSSION

In present study, the pregnant rats treated orally with dexamethasone from 5th day of gestation to 20th day of gestation didn't report any indication of abortion or dead cases. There was a considerable decrease in corrected body weight of treated mother when compared with control and fennel oil groups. The decrease in maternal body weight could be due to reduced food consumption or due to the developmental toxicity of the drug used or may be due to maternal body metabolic disorders (Kassem, 2013). Our work showed decrease in average weight of uterus, high incident of resorption, pre-implantation and post implantation loss index was increased in treated group with dexamethasone as compared with control and fennel oil groups, the decrease in average weight of the uterus maybe linked with increase resorption site. Also, there was a significant decrease in placenta weight of treated mother when compared with control and fennel oil groups. In present study, the oral administration of pregnant rat with dexamethasone leads to significant decrease in the number and weight of viable fetus and decreased in number of live birth fetuses. The decrease in the number of viable fetuses and their weights may be due to the accumulation of drugs in the fetus body instead of the maternal body, which enhanced by the very simplified structure of rats' placenta that allowed drugs to pass out of their circulation and accumulated in the fetal tissues or function as inhibitors of membrane enzymes involved in embryonic nutrition (Tuchman, 1975). Dexamethasone decreased the weights of fetuses as it was observed very early in the history of the development of corticosteroids that small doses suppressed growth in the rat (Wells *et al.*, 1940). Hematoma can result because dexamethasone reduces subcutaneous collagen and cause atrophic changes in the skin, resulting in fragile skin and hematoma. Purpura has been observed during glucocorticoid treatment and an increased fragility of the capillaries is thought to occur in about 60% of these patients

(Aronson, 2006). Different glucocorticoid with very different potencies can have different effects on cartilage cells and native tissues, as well as in various animal models (Sadowski and Steinmeyer, 2001; Mushtaq and Ahmed, 2002; Siengdee *et al.*, 2015; Dragoo *et al.*, 2012). We have found that addition of dexamethasone results in increase osteoclast formation and bone resorption (Van de Wijngaert *et al.*, 1987). Also, in our study the group treated with dexamethasone revealed incomplete ossification of frontal, nasal, mandible and parietal, the sternum showed incomplete ossified sternebra. Also, the ribs anomalies represented in the wavy ribs, curved ribs and costal separation. The delayed ossification and abnormalities of the ribs and vertebrae is in accordance with the results of (Van de Wijngaert *et al.*, 1987; Sadowski and Steinmeyer, 2001; Mushtaq and Ahmed, 2002; Dragoo *et al.*, 2012; Siengdee *et al.*, 2015). In present study, the pregnant rats treated with dexamethasone and fennel oil revealed increase in corrected body weight of pregnant rats, increase average weight of placenta, increase average weight of uterus, decreased in preimplantation and post implantation loss index,

increase in number and weight of viable fetuses, and decrease in average number of hematomas in compared with dexamethasone group. Our work showed completely ossified bones of maxilla, mandible, parietal, frontal, also there was complete ossification of bones of ribs and vertebral column, sternum, pectoral girdle and forelimb, pelvic girdle and hind limb. Besides, there was no ribs anomalies were detected. The liver plays important roles in homeostasis, including metabolism, glycogen storage, bile secretion, production of various serum proteins, and drug detoxification, in cases of acute liver injury, hepatic cells, especially hepatocytes, can rapidly proliferate to repair damage (Suzuki *et al.*, 2002). The present study the fetal liver maternally treated with dexamethasone showed loss of normal architecture, pyknosis of the nucleus, congested central vein and dilated sinusoids. In addition, hemorrhage area, necrotic area and fatty degeneration were observed. In accordance with this result (Noel, 2013; Shama *et al.*, 2013) reported dexamethasone show cytoplasmic fatty vacuolation and necrosis of the hepatocyte and central vein congestion, expansion of sinusoids, and morphological changes in hepatic tissue is caused by the side effects of dexamethasone. In the current study, the fetal liver maternally treated with dexamethasone and fennel oil showed histological improvement, where the hepatocytes become more organized, normal branching cords of hepatocytes around the central vein separated by blood sinusoids were found, also the endothelial lining the central vein showed normal appearance. In agreement with these result (Al-Amoudi, 2017) reported that the adult male albino rats treated orally with fennel oil attenuate the histopathological changes induced by sodium valporic drug and revealed nearly normal appearance of hepatic tissues. In addition, the essential oils of fennel have hepatoprotective effects (Ozbek *et al.*, 2003).

CONCLUSION

This study revealed that the use of fennel oil (1 ml/kg) during gestation period of pregnant albino rats have important ameliorative effect against embryotoxicity of dexamethasone. Future studies should be carried out in order to determine and understand the mechanism of action by which the fennel oil could ameliorate the teratogenic effect induced by dexamethasone.

CONFLICT OF INTEREST

Author stated that there is no conflict of interest.

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EXPLANATIONS OF FIGURES

Fig. 1 A histogram showing effect of fennel oil, dexamethasone, and fennel oil with dexamethasone on mother correct body weight (CBW), uterus weight

(U Wt.), fetus weight (F Wt.) and Placenta weight (P Wt) at 20th day of gestation.

a letter means there was a significant difference ($p < 0.05$) as compared with

Control group.

b letter means there was a significant difference as compared with Oil group.

c letter means there was a significant difference as compared with dexamethasone group.

d letter means there was a significant difference as compared with dexamethasone + Oil group.

Fig. 2 Photographs of uterus of control pregnant rat at the 20th day of gestation. A showing normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina, P= placenta, F= fetuses.

Fig. 3. Photographs of uterus of pregnant rat treated with the 1ml/kg fennel oil at the 20th day of gestation. A Showing: normal symmetrical distribution of fetuses in the two uteri horns.

Fig. 4 Photographs of uterus of pregnant rat treated with dexamethasone at the 20th day of gestation. U= Uterus, V= Vagina, P= placenta, F= fetuses, RS= resorption sites. Showing: A, B: Asymmetrical distribution of fetuses in the two uteri horns and resorption sites

Fig. 5 A Photograph of uterus of pregnant rat treated with dexamethasone and the 1ml/kg fennel oil at the 20th day of gestation. Showing: normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina, P= placenta, F= fetuses.

Fig. 6 A histogram showing Resorption Number, implantation loss index (Post), preimplantation loss index, Number of viable fetus (VF), corpora lutea (CL) and Number of Hematoma at 20th of gestation..

a letter means there was a significant difference ($p < 0.05$) as compared with Control group.

b letter means there was a significant difference as compared with Oil group.

c letter means there was a significant difference as compared with dexamethasone group.

d letter means there was a significant difference as compared with dexamethasone + Oil group.

Each value represented as Means \pm SEM.

- Fig. 7** Photographs of fetuses of control mother at 20th day of gestation. Showing: (A, B) All Fetuses exhibited normal morphology and normal length.
- Fig. 8** Photographs of fetuses of maternally treated with fennel oil. Showing: (A, B) Fetuses have normal morphology and normal length.
- Fig. 9** Photographs of fetuses of maternally treated with dexamethasone at 20th day of gestation. Showing: A) Fetus with dorsal Hematoma and visceral hematoma.
- B) Fetus with deformed fore limb (DL) and unusual shape (US) and short neck.
- C) Fetus with deformed fore limb and unusual small shape.
- D) Fetus visceral hematoma.
- E) Fetus with hematoma at fore limb.
- F) Small in size and shortness of the neck and unusual shape.
- Fig. 10:** Photographs of Fetuses maternally treated with dexamethasone and the fennel oil at the 20th day of gestation. Showing: All Fetuses exhibited normal morphology. No abnormalities / deformations were observed.
- Fig. 11** Photographs of the control fetal skeleton (Alcian blue & Alizarin red stain). Showing: A: ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparieta. B: complete ossification of ribs. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L. V= lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification of pectoral girdle and forelimbs. Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna,

R= radius, MC= metacarpals. E: complete ossification of pelvic girdle and hindlimbs. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

Fig. 12 Photographs of the fetal skeleton treated with fennel oil (Alcian blue & Alizarin red stain). Showing: A: complete ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparieta. B: complete ossification of ribs. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L.V = lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification. Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpals. E: complete ossification. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

Fig. 13 Photographs of the skeleton from treated group with dexamethasone. Showing:

A: Less ossified Frontal (Fr) and Parietal (Pr), Nasal (Na) maxilla and Mandible. B: Wavy ribs (short arrow) and costal separation (long arrow). C: Curved ribs. D: Incomplete ossification of sternbrae bones. E: less ossified Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpal. F: less ossification. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

Fig. 14 Photographs of the fetal skeleton treated with fennel oil and dexamethasone (Alcian blue & Alizarin red stain). Showing: A: complete ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparietal. B: complete ossification. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L.V = lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification of Pectoral girdle and Forelimb Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpals. E: complete ossification of Pelvic girdle and Hind limb I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus

Fig. 15 Photomicrographs of a section of fetal liver of control pregnant rat. H&E stain. Showing (A&B): normal structure of the liver tissue. The central vein with its intact endothelial lining (CV), hepatocytes (black arrow), blood sinusoids (S) and numerous of erythroblasts (red arrow)

Fig. 16 Photomicrographs of a section of fetal liver of pregnant rat treated with fennel oil. H&E stain. Showing (A&B): common construction of the liver tissue. The central vein (CV), Hepatocytes (black arrow), plentiful erythroblasts (red arrow) and blood sinusoids (S).

Fig. 17 Photomicrographs of a section of fetal liver of pregnant rat treated with dexamethasone. H&E stain. Showing: A: loss normal architecture of hepatic tissue, empty areas (red star) are scattered all over the tissue, rupture of epithelial cells lining central vein (CV), hemorrhage (black star) and multiple number of erythroblasts (black arrow) can be seen. B: Disorganization of hepatic tissue, empty areas (red star) can be seen, rupture of epithelial cells lining central vein (CV), degenerated hepatocytes (red arrow).

C: Disorganization of hepatic tissue, empty areas (red star) can be seen, degenerated hepatocytes (red arrow), karyolysis nuclei (green arrow). D: Disorganization of hepatic tissue, empty areas (red star) can be seen, degenerated hepatocytes (red arrow), karyolysis nuclei (green).

Fig. 18 Photomicrographs of a section of fetal liver of pregnant rat treated with fennel oil and dexamethasone. H&E stain. Showing: A - C: good appearance of the hepatic tissue. The central vein (CV), hepatocytes (black arrow), blood sinusoids (S) and numerous of erythroblasts (red arrow).

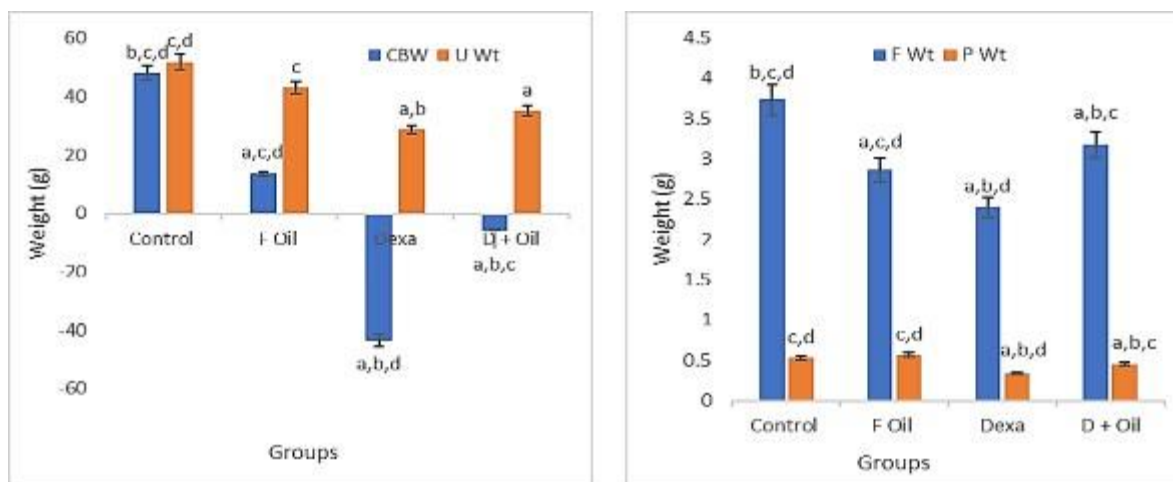


Fig. 1: A histogram showing effect of fennel oil, dexamethasone, and fennel oil with dexamethasone on mother correct body weight (CBW), uterus weight (U Wt.), fetus weight (F Wt.) and Placenta weight (P Wt.) at 20th day of gestation.

a letter means there was a significant difference ($p < 0.05$) as compared with Control group.
b letter means there was a significant difference as compared with Oil group.

c letter means there was a significant difference as compared with dexamethasone group.

d letter means there was a significant difference as compared with dexamethasone + Oil group.

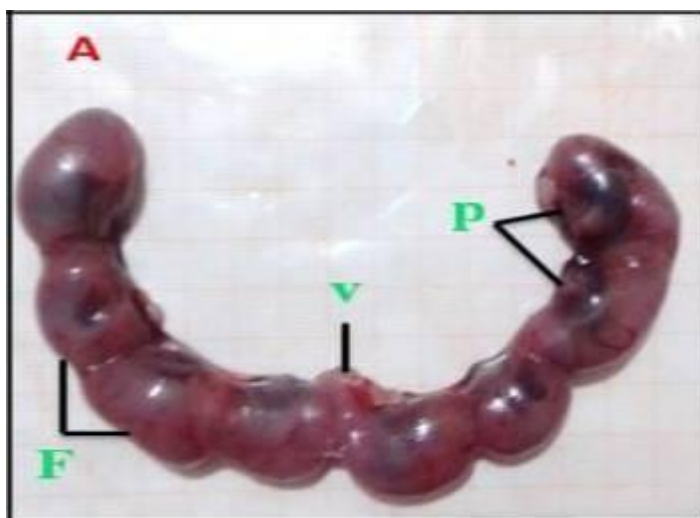


Fig. 2: Photographs of uterus of control pregnant rat at the 20th day of gestation. A showing normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina, P= placenta, F= fetuses.

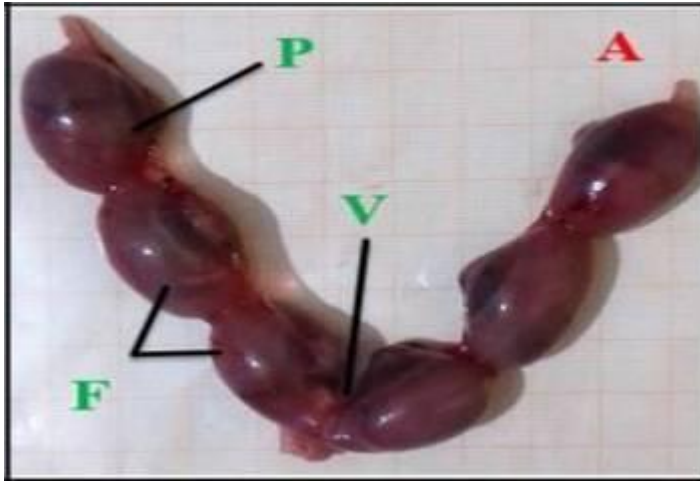


Fig. 3: Photographs of uterus of pregnant rat treated with the 1ml/kg fennel oil at the 20th day of gestation. A Showing: normal symmetrical distribution of fetuses in the two uteri horns.

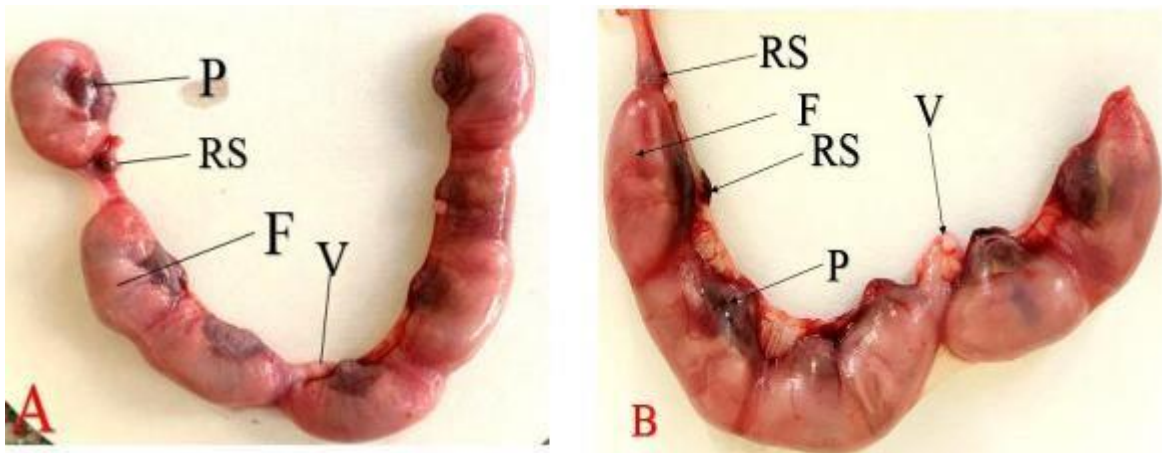


Fig. 4: Photographs of uterus of pregnant rat treated with dexamethasone at the 20th day of gestation. U= Uterus, V= Vagina, P= placenta, F= fetuses, RS= resorption sites. Showing: A, B: Asymmetrical distribution of fetuses in the two uteri horns and resorption sites



Fig. 5: A Photograph of uterus of pregnant rat treated with dexamethasone and the 1ml/kg fennel oil at the 20th day of gestation. Showing: normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina, P= placenta, F= fetuses.

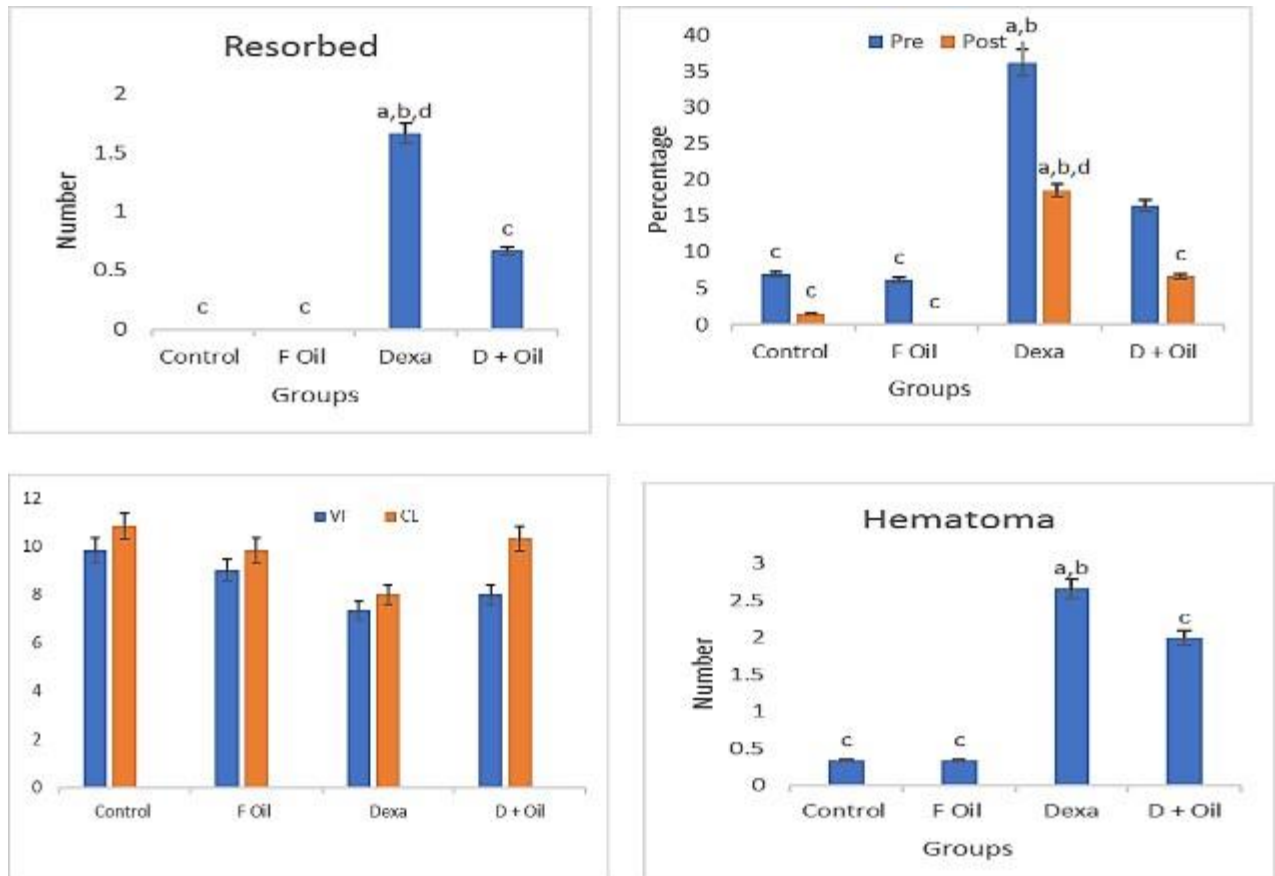


Fig. 6: A histogram showing Resorption Number, implantation loss index (Post), preimplantation loss index, Number of viable fetus (VF), corpora lutea (CL) and Number of Hematoma at 20th of gestation..

a letter means there was a significant difference ($p < 0.05$) as compared with Control group. b letter means there was a significant difference as compared with Oil group.

c letter means there was a significant difference as compared with dexamethasone group.

d letter means there was a significant difference as compared with dexamethasone + Oil group.

Each value represented as Means \pm SEM.

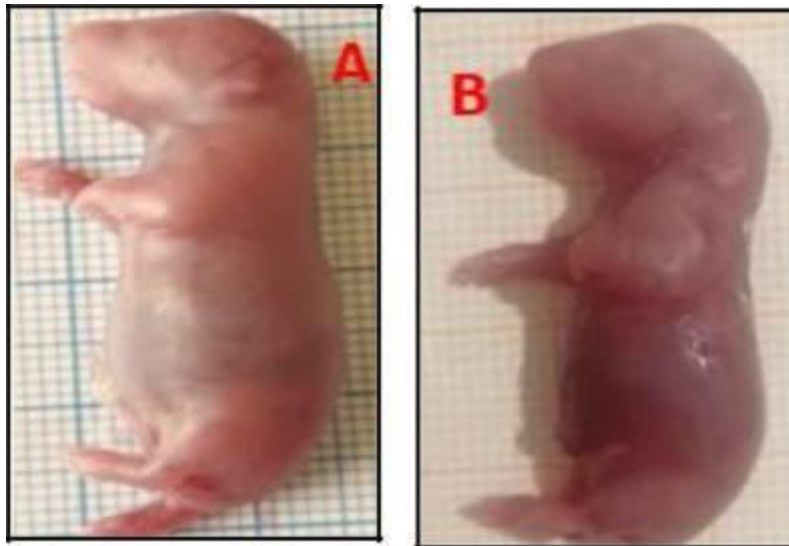


Fig. 7: Photographs of fetuses of control mother at 20th day of gestation. Showing: (A, B) All Fetuses exhibited normal morphology and normal length.



Fig. 8: Photographs of fetuses of maternally treated with fennel oil. Showing: (A, B) Fetuses have normal morphology and normal length.

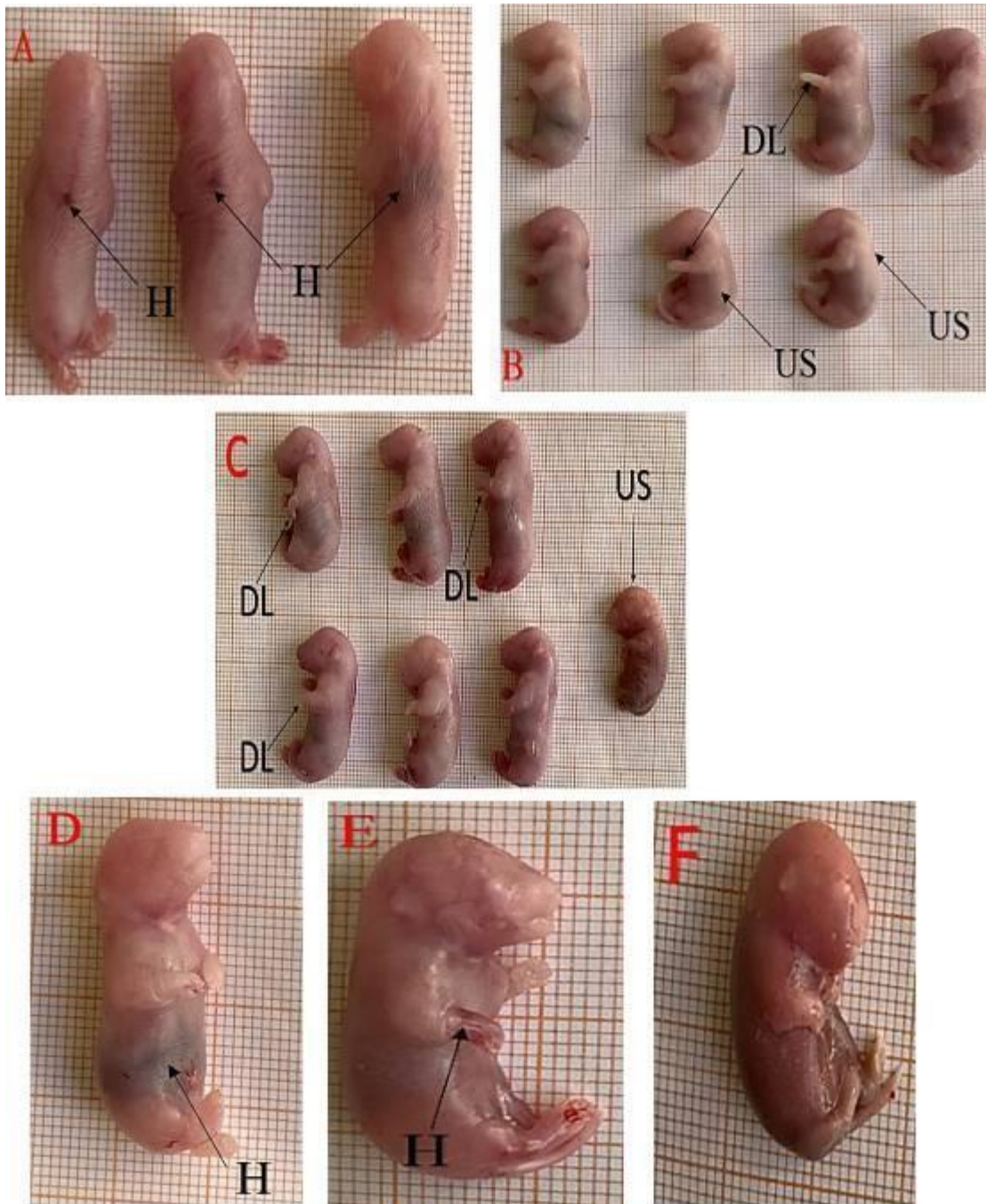


Fig. 9: Photographs of fetuses of maternally treated with dexamethasone at 20th day of gestation. Showing: A) Fetus with dorsal Hematoma and visceral hematoma. B) Fetus with deformed fore limb (DL) and unusual shape (US) and short neck. C) Fetus with deformed fore limb and unusual small shape. D) Fetus visceral hematoma. E) Fetus with hematoma at fore limb. F) Small in size and shortness of the neck and unusual shape.



Fig. 10: Photographs of Fetuses maternally treated with dexamethasone and the fennel oil at the 20th day of gestation. Showing: All Fetuses exhibited normal morphology. No abnormalities / deformations were observed.

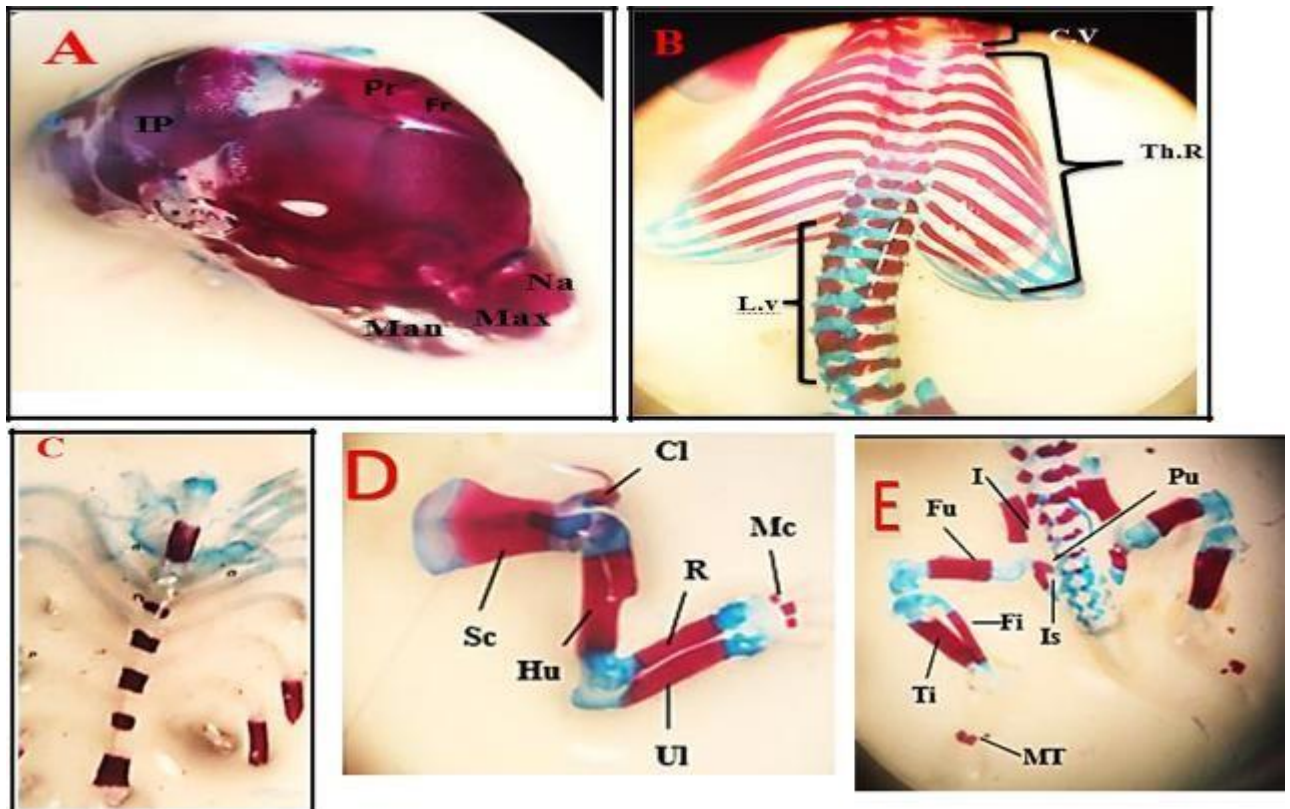


Fig. 11: Photographs of the control fetal skeleton (Alcian blue & Alizarin red stain). Showing: A: ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparieta. B: complete ossification of ribs. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L. V= lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification of pectoral girdle and forelimbs. Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpals. E: complete ossification of pelvic girdle and hindlimbs. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

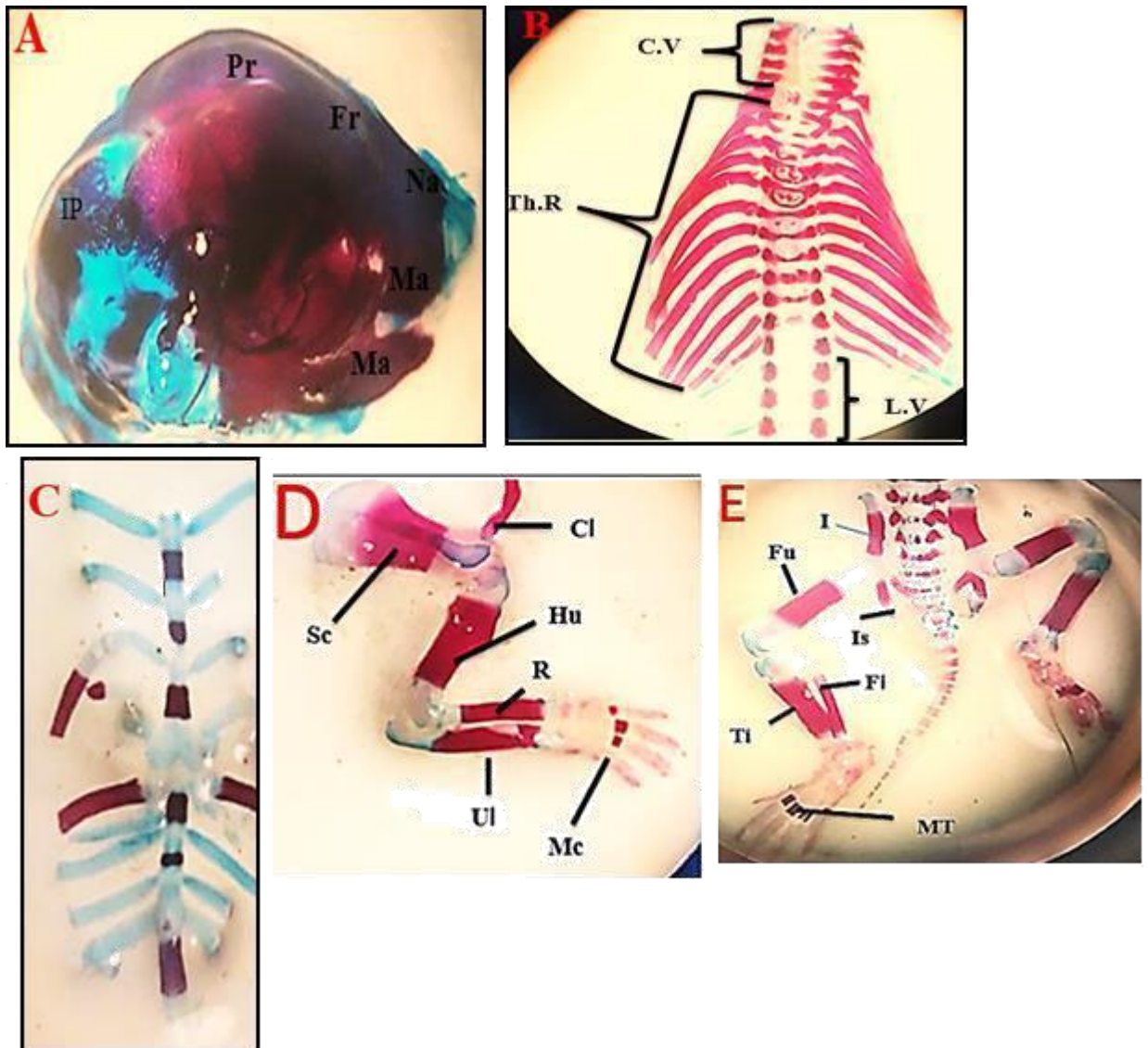
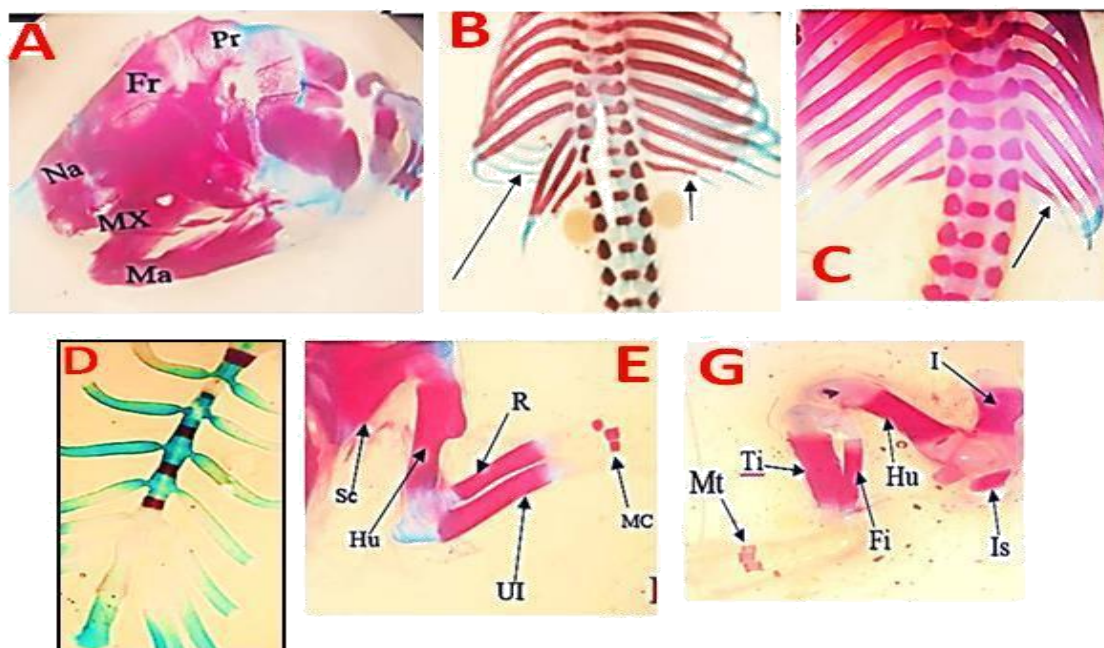


Fig. 12: Photographs of the fetal skeleton treated with fennel oil (Alcian blue & Alizarin red stain). Showing: A: complete ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparieta. B: complete ossification of ribs. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L.V = lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification. Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpals. E: complete ossification. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

Fig. 13:



Photographs of the skeleton from treated group with dexamethasone. Showing:

- A: Less ossified Frontal (Fr) and Parietal (Pr), Nasal (Na) maxilla and Mandible.
- B: Wavy ribs (short arrow) and costal separation (long arrow).
- C: Curved ribs.
- D: Incomplete ossification of sternbrae bones.
- E: less ossified Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpal.
- F: less ossification. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

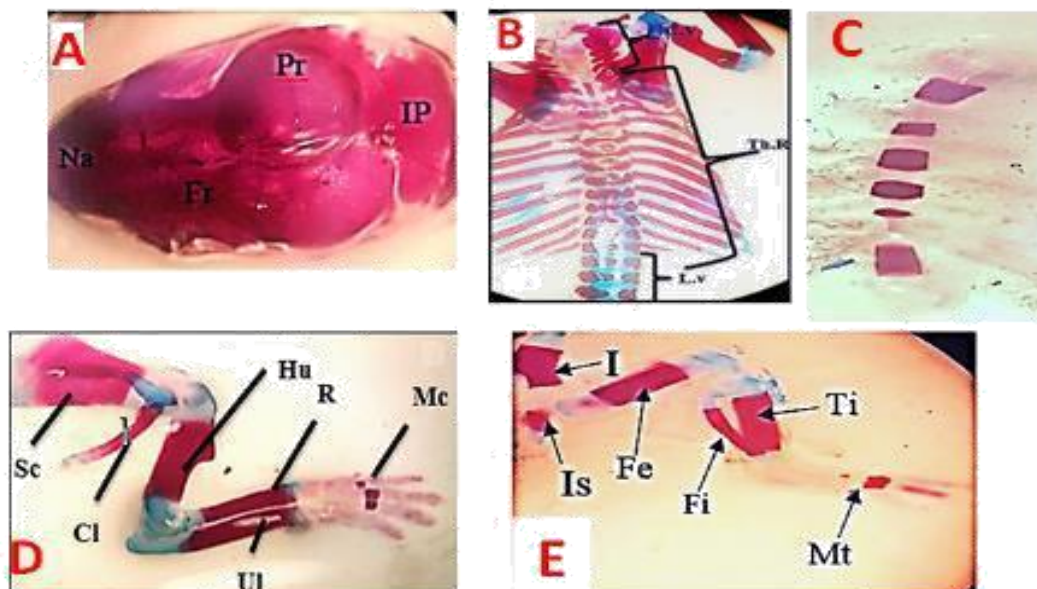
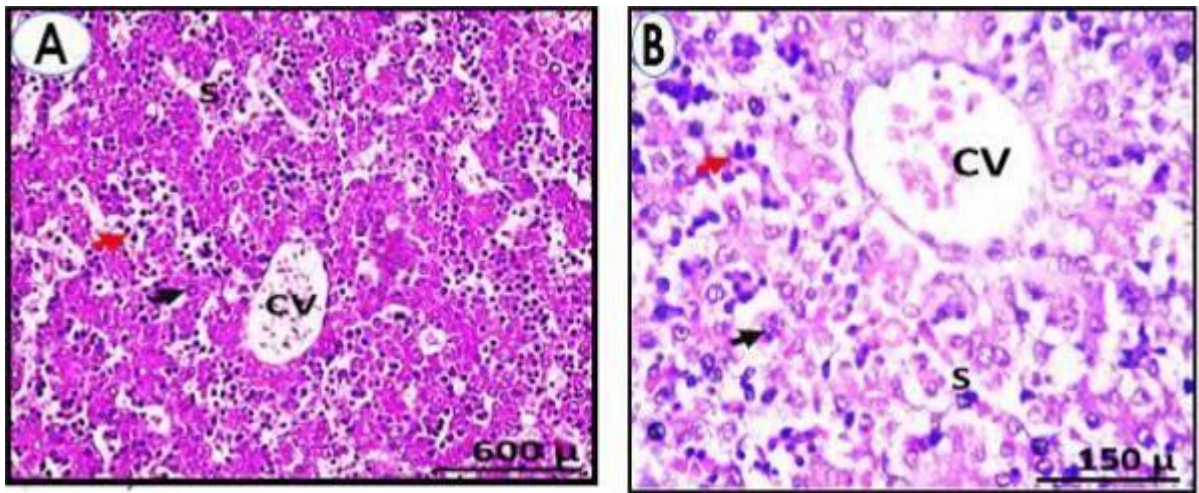


Fig. 14: Photographs of the fetal skeleton treated with fennel oil and dexamethasone (Alcian blue & Alizarin red stain). Showing: A: complete ossification of the cranial bones. Fr= frontal, Pr= parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparietal. B: complete ossification. C. V= cervical vertebrae, Th V= thoracic vertebrae, Th R= thoracic rib, L.V = lumbar vertebrae. C: complete ossification of sternbrae bones. D: complete ossification of Pectoral girdle and Forelimb Cl= clavical, Sc= scapula, Hu= humerus, Ul= ulna, R= radius, MC= metacarpals. E: complete ossification of Pelvic girdle and Hind limb I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus

The histological study of fetal liver

Fig. 15: Photomicrographs of a section of fetal liver of control pregnant rat. H&E stain.



Showing (A&B): normal structure of the liver tissue. The central vein with its intact endothelial lining (CV), hepatocytes (black arrow), blood sinusoids (S) and numerous of erythroblasts (red arrow).

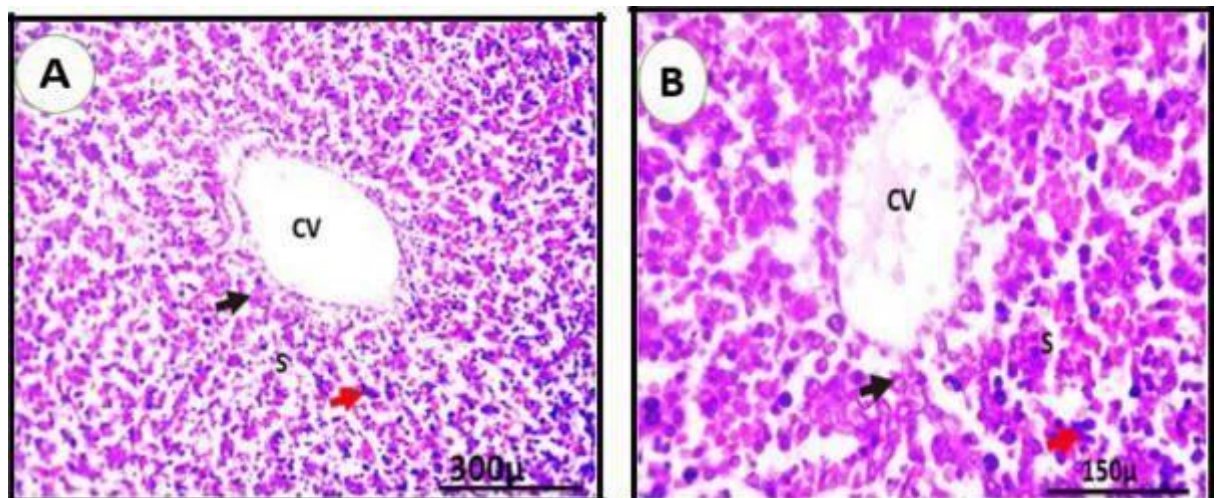


Fig. 16: Photomicrographs of a section of fetal liver of pregnant rat treated with fennel oil. H&E stain. Showing (A&B): common construction of the liver tissue. The central vein (CV), Hepatocytes (black arrow), plentiful erythroblasts (red arrow) and blood sinusoids (S).

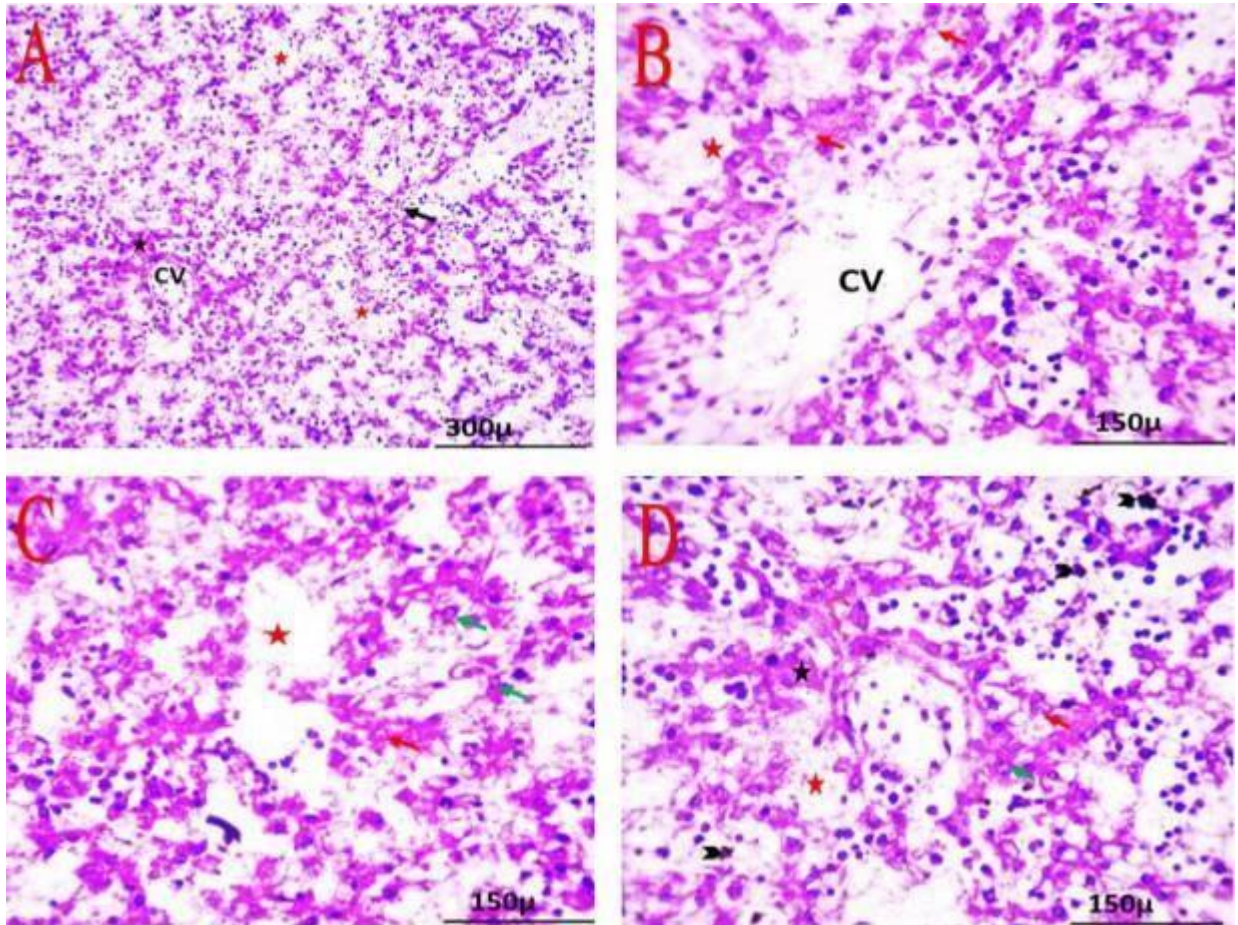


Fig. 17: Photomicrographs of a section of fetal liver of pregnant rat treated with dexamethasone. H&E stain. Showing: A: loss normal architecture of hepatic tissue, empty areas (red star) are scattered all over the tissue, rupture of epithelial cells lining central vein (CV), hemorrhage (black star) and multiple number of erythroblasts (black arrow) can be seen. B: Disorganization of hepatic tissue, empty areas (red star) can be seen, rupture of epithelial cells lining central vein (CV), degenerated hepatocytes (red arrow). C: Disorganization of hepatic tissue, empty areas (red star) can be seen, degenerated hepatocytes (red arrow), karyolysis nuclei (green arrow). D: Disorganization of hepatic tissue, empty areas (red star) can be seen, degenerated hepatocytes (red arrow), karyolysis nuclei (green).

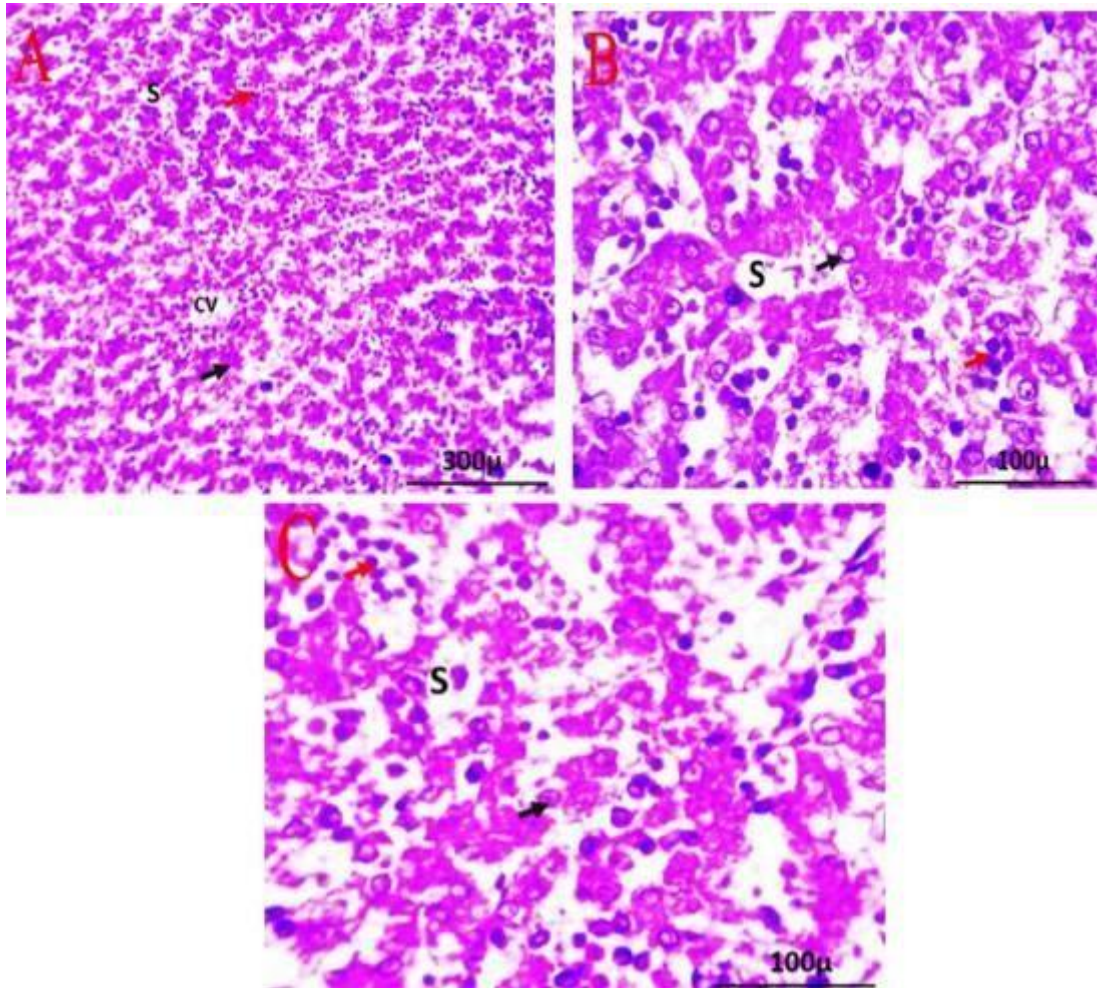


Fig. 18: Photomicrographs of a section of fetal liver of pregnant rat treated with fennel oil and dexamethasone. H&E stain. Showing: A - C: good appearance of the hepatic tissue. The central vein (CV), hepatocytes (black arrow), blood sinusoids (S) and numerous of erythroblasts (red arrow).

Table (1): Showing Pregnancy Outcomes.

Groups	Control	Oil	Dexamethasone	Dexamethasone + Oil

No. pregnant	6	6	6	6
Corpora Lutea number/Litter	10.83±0.542	9.83±1.47	8.0±1.59	10.33±0.21
No. resorbed/litter	0±0.00 ^c	0±0.00 ^c	1.66±0.21 ^{a,b,d}	0.66±0.42 ^c
Pre-implantation loss index-%	6.90±4.36 ^c	6.16±4.90 ^c	36.20±8.21 ^{a,b}	16.37±2.29
Post-implantation loss index-%	1.5±1.5 ^c	0±0.00 ^c	18.53±2.28 ^{a,b,d}	6.66±4.21 ^c
No. litters with viable fetuses	6	6	6	6
No. litter with resorption	0	0	4	2
No. litter with complete resorption	0	0	0	0
No. viable fetuses/litter	9.83±0.477	9.0±1.34	7.33±0.557	8.0±0.00
Gravid uterine weight (g)	51.72±2.18 ^{c,d}	42.97±5.02 ^c	28.67±2.01 ^{a,b,d}	34.97±1.69 ^a
Corrected wt. gain (g)	47.95±2.81 ^{b,c,d}	13.65±6.82 ^{a,b,c}	-43.6±4.90 ^{a,b,d}	-5.70±1.95 ^{a,b,c}
Body weight of fetuses (g)	3.73±0.057 _{b,c,d}	2.86±0.033 ^{a,c,d}	2.40±0.07 ^{a,b,d}	3.17±0.06 ^{a,b,c}
Placenta weight (g)	0.526±0.011 ^{c,d}	0.533±0.015 ^{c,d}	0.335±0.018 ^{a,b,d}	0.454±0.014 ^{b,c}
No. of fetuses having hematoma/Litter	0.333±0.333 ^c	0.333±0.333 ^c	2.66±0.55 ^{a,b}	2.0±0.63

Each value represented as Means ± SEM.

a letter means there was a significant difference ($p < 0.05$) as compared with Control group.

b letter means there was a significant difference as compared with Oil group.

c letter means there was a significant difference as compared with Dexa group.

d letter means there was a significant difference as compared with Dexa + Oil group.

Skeletal anomalies:

Table (2): Showing Fetal Ribs Anomalies at 20th GD.

<u>Groups</u>	<u>Control</u>	<u>Fennel oil</u>	<u>Dexamethasone</u>	<u>Dexamethasone + fennel oil</u>
	35 — Pairs	30 Pairs	23 Pairs	30 Pairs
<u>Wavy rib</u>	0(0)	0(0)	38(82.6)	22(36.6)
<u>Curved rib</u>	0(0)	0(0)	12(26.1)	10(16.7)
<u>Rudimentary</u>	0(0)	0(0)	0(0)	0(0)
<u>Costal separation</u>	0(0)	0(0)	14(30.4)	0(0)

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