THE PROTECTIVE EFFECT OF COQ10, DHEA AND THEIR COMBINATION IN REDUCED EMBRYOTOXICITY AND TERATOGENICITY FOR NORFLOXACIN IN PREGNANT FEMALE RATS

Bassim Kh. Al-Rekabi * , Muhammed A. Al-Diwan ** , Alaa A. Sawad ***

* Department of Animal production, College of Agriculture, University of Summer, Thi-Qar, Iraq.

** Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

*** Department of Anatomy, Histology and Embryology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

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Corresponding authors: kh_bassim@yahoo.com

ABSTRACT

This study was designed to evaluate the protective effect of CoQ10 and DHEA and their combination on norfloxacin induced embryotoxicity and teratogenicity in pregnant female rats and their fetuses. Twenty pregnant rats were divided equally into 5 groups, 4 animals each group as following: Control group (G1): 4 normal pregnant female received orally DMSO 0.5ml/animal/day from 5^{th} - 19^{th} day of gestation, first treated group (T1): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily, second treated group (T2): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with CoQ10 200 mg/kg IP, third treated group (T3): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with DHEA 25 mg/kg IP, and fourth treated group (T4): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with a combination of CoQ10 200 mg/kg and DHEA 25 mg/kg IP for the same period. Norfloxacin was administered by oral gavages as a single dose. All dams were sacrificed at 20^{th} day of gestation and their fetuses were collected and subjected to morphological and skeletal examinations. The obtained results showed that exposure to norfloxacin in pregnant female rats during gestational period from 5^{th} -19^{th} day demonstrated a significant increased ($P \leq 0.05$) in resorbed and stillbirth fetuses (dead fetuses at birth), and a

significant decrease in fetal body weight, fetal body length, fetal tail length, maternal weight gain and placental weight; and also caused skeletal malformation in all cranial bone compared to control group. It has been concluded that CoQ10 and DHEA prevented and treated morphological and skeletal anomalies in rat fetuses. Therefore, CoQ10 and DHEA are potential therapeutic antioxidant agents against fetotoxicity and teratogenicity induced by oxidative stress generated by norfloxacin intoxication.

INTRODUCTION

Exposure of developing embryo or fetus to definite chemical agents and drugs is known to produce congenital anomalies leading to death in uterine or structural birth defects usually known as teratogenesis [1]. Different forms of embryonic malformation have been attributed to oxidative stress [2]. There has been a forceful evidence involving teratogens like norfloxacin to reactive oxygen species generation [3]. Norfloxacin (NFX) is an active semi-synthetic chemotherapeutic antibacterial agent and has a broad spectrum of antibacterial activity against Gram negative and Gram-positive aerobic bacteria [4]. It is a therapeutic agents were able to cross placental barrier and enter fetal circulation. It act by inhibition bacterial DNA replication by inhibition of bacterial DNA gyrase and topoisomerase II enzyme [5]. Every agents given during pregnancy therefore has a tendency to produce some sort of structural abnormality in neonate at birth until proved otherwise [6]. However, period of organogenesis is mainly serious stage for malformation to occur. Agents given during this period are more likely to cause birth defects. This critical time of fetal developments in rats and mice is from 6-15 days of their gestation [7]. A birth defect or a congenital abnormalities is a structural malformation of any type current at birth, which may be macroscopic or microscopic, on surface or within body [8]. The fetal risks of these substances occurs during pregnancy by impaired cartilage formation in animal studies [9]. Coenzyme Q10 (CoQ10) is an endogenous substance act as a vital antioxidant proposed for cellular membrane integrity either by direct reaction with free radicals or by regeneration other antioxidant [10]. It is a lipid soluble, vitamin like substance required for proper functioning of many organs and chemical reactions in body [11]. It has many beneficial effects in human and animals health including cardiovascular disease, neurodegenerative disorders, age related disorders, autoimmune disorder, DNA damage, thyroid disorders, male infertility, cancers, diabetes, fibrosis, apoptosis, and obesity. It is a crucial redox and proton translocations constituent of mitochondrial respiratory chain, play an essential role in mitochondrial energy production through redox activity in the electron transport chain, transporting electrons between enzymes. Thus, it is plays an essential role in cellular

bioenergetics and membrane stabilizer and production of ATP in oxidative respiration process [12]. [13] demonstrated that CoQ10 has anti-inflammatory properties decreasing production of pro-inflammatory cytokines such as interleukin (IL) and tumor necrosis factor (TNF-α). Dehydroepiandrosterone (DHEA) is one of the most abundant endogenous circulating steroid hormone with multi-functional properties, it is produced in adrenal glands, gonads, and brain, where it functions as a metabolic intermediate in biosynthesis of androgen and estrogen sex steroids [14]. It plays a critical endogenous antioxidant and pro-oxidant activity. It can also protect against lipid peroxidation (LPO) induced by oxidative damage [15]. DHEA also have anti-inflammatory properties through reduced pro-inflammatory cytokines secretion like IL and TNF-α and regulation of body immune response [16]. DHEA and DHEAS are products of cholesterol metabolism with first enzymatic reaction occurs in mitochondria and are resulting from the action of cytochrome P450 [17]. This study aimed to evaluate the ameliorative effect of CoQ10 and DHEA and their combination on embryotoxicity and teratogenicity induced by norfloxacin in pregnant female rats.

MATERIAL AND METHODS

Drugs and chemical reagents

Norfloxacin was obtained as an tablets from Ajanta Pharma limited, India, 400 mg tablets) under a trade name (Norexin) and administered by oral gavages as a single dose, and CoQ10 200 mg/kg and DHEA 25mg/kg obtained from (Sigma, St. Louis, MO, USA) was administered intraperitoneally. Dimethylsulphoxide (DMSO) was purchased from Merck, Darmstadt, Germany.

Experimental animals

Thirty male and female healthy rats (*Rattus norvegicus*) weighing 225-250 grams, 12-14 weeks old (10 male Vs. 20 female) were randomly divided into five equal groups, each group consisted of 6 rats (2 Male Vs. 4 female). Each 6 animal was housed in an individual cage measured as15 x 35 x 50 cm and kept under normal temperature 22 - 28 °C and the daily light period was 12 hours by use of two fluorescent lamps, and humidity rate was about 50 %. Animals were provided with water and diet *ad libitum.* The sexually mature female rats were acclimatized in laboratory for 2 weeks, followed with daily vaginal smear examination for 4 pre-treatment estrous cycles as described by [18] to establish their normal pattern of cyclical activity. The

female rats with proestrus stage were caged overnight and mated with fertile normal healthy males of same strain, allowing one male for two females in one cage [19]. On next morning, the female rats were examined for signs of mating such as sperms in vaginal smears or a vaginal plug of mucoid greenish white material. Presence of both or any of these signs was considered as day-1 of pregnancy [20].

Experimental design and study strategy

After detection the first day of gestation for all females, 20 animals had been divided randomly and equally into 5 groups as following: Control group (G1): 4 normal pregnant female received orally DMSO 0.5ml/animal/day from 5^{th} - 19^{th} day of gestation, first treated group (T1): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily, second treated group (T2): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with CoQ10 200 mg/kg IP, third treated group (T3): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with DHEA 25 mg/kg IP, and fourth treated group (T4): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hours injected daily with a combination of CoQ10 200 mg/kg and DHEA 25 mg/kg IP for same period of treatment.

Developmental observations

The rats before sacrifice were first weighed and then anaesthetized by placing them in a closed beaker containing cotton sucked with chloroform for anesthesia. The abdominal cavity was opened up through a midline abdominal incision to take the fetuses at 20^{th} day of gestation. After abdominal incision, the uterus was observed and location and number of the fetuses and resorption sites were observed. Fetal body weight, fetal body length (CRL), and fetal tail length were then evaluated. On 20^{th} day of gestation, all pregnant female rats of groups were sacrificed and fetuses were removed from the uterus and evaluated for fetal mortality rate (resorped or dead fetuses), and living fetuses were recorded. Fetal growth parameters, morphological and skeletal malformation were also recorded.

Alizarin red and Alcian blue skeletal staining

Fetuses were preserved in 95% ethyl alcohol for 12-48 hours and rocked slowly at room temperature and were stained with double staining of fetal skeletons for cartilage (Alcian blue) and bone (Alizarin red) according to the method described by [21]. The fetuses were examined carefully for external anomalies. Then, the fetuses where stained in a mixture of 0.14% alcian blue and 0.12% alizarin red stain in ethanol and glacial acetic acid. Fetuses were then macerated

in 2.00% KOH for 12-24 hours, cleared and hardened in 1:1 glycerol 95% ethanol for 1day and distilled water, and stored in pure glycerin and investigated by dissecting microscope.

Statistical Analysis

In this study, one way ANOVA analysis and LSD tests are used according to (IBM SPSS, version 20) program at the ($P \leq 0.05$) to find the means for all treatments (IBM SPSS, 2011).

RESULTS

Maternal rats toxicity

Maternal weight gain and placental weight changes

The exposure pregnant female rats to norfloxacin dose (400 mg/kg) during gestational period from 5^{th} -19^{th} day revealed a significant decrease ($P \leq 0.05$) in maternal weight gain and placental weight changes compared to control group (Table2). Whereas, the groups that treated with CoQ10, DHEA and their combination revealed significantly increase ($P \leq 0.05$) in maternal weight gain and placental weight changes compared to groups treated with norfloxacin, but they were still less significantly ($P \leq 0.05$) compared with control value. It is also observed from table (2) that the combination the of CoQ10 and DHEA caused a highly significant increased ($P \leq 0.05$) in maternal weight gain and placental weight changes and almost return to its normal levels compared with control value.

Morphological examination of uterus

The results in figure (3) demonstrated that the pregnant female rats received norfloxacin (400mg/kg) on 20^{th} day of gestation showed completely resorbed uterus, clearly visible embryonic resorption, decrease in number of implanted fetuses, and abnormal shape with asymmetrical distribution of the fetuses in two uteri horn compared to control group. Whereas, the groups that treated with CoQ10, DHEA and their combination showed normal shape, weight and length with symmetrical distribution of the fetuses between the two uterine horns (figure 4,5 and 6 respectively) compared to group treated with norfloxacin intoxication.

Effect of norfloxacin (NFX) on developing fetus

Growth retardation rate

The results on table (2) and figure (7) also pointed out that the pregnant female rats received norfloxacin showed significantly decreased (P≤ 0.05) in fetal body weight, fetal body length and fetal tail length compared to control group. Whereas, the groups treated with CoQ10, DHEA and combination of CoQ10 and DHEA showed a significant increased (P≤ 0.05) in fetal body weight, fetal body length and fetal tail length compared to groups treated with norfloxacin, but they were still less significantly (P≤0.05) compared to control value. It was indicated from table (2) and figure (7) that combination of CoQ10 and DHEA caused a highly significant increased in growth retardation rate represented by fetal body weight, fetal body length and fetal tail length, and almost return to its normal levels compared with control value.

3.2.1.2. Total prenatal mortality rate

The results in table (1) and figure (3) also illustrated a significant increased (P≤ 0.05) in resorbed and dead fetuses of pregnant female rats at 20th day of gestation that received norfloxacin compared to control group. Whereas, the groups treated with CoQ10, DHEA and combination of CoQ10 and DHEA showed a significant decreased (P≤ 0.05) in resorbed and dead fetuses on day 20th of gestation compared to groups treated with norfloxacin, but they still less significantly (P≤ 0.05) compared to the control value. It is also observed from table (1) and figure (4, 5, and 6) that combination of CoQ10 and DHEA caused a highly significant decreased in resorbed and stillbirth and almost return to its normal levels compared with control value.

Skeletal examination of fetuses at 20th day of gestation

Axial skeleton of fetuses at 20th day of gestation

Bones of fetus skull roof (cranial bones)

The results on figure (1) and table (3) showed that exposure to norfloxacin (400mg/kg) to pregnant female rats at 20th day of gestation showed incomplete ossification of all fetal cranial bones, interparietal, parietals, frontals, nasal, mandible and maxilla compared to control group. Whereas, the groups that treated with CoQ10, DHEA and combination of CoQ10 and DHEA showed complete ossification of all fetal cranial bones compared to group treated with norfloxacin, but they were still less ossified compared to control group. It is also observed from the figure (1) and table (3) that the combination of CoQ10 and DHEA caused a highly ossified in all fetal cranial bones compared to control group.

Table (1): The effect of CoQ10 and DHEA and their combination on fetal mortality rate represented by resorped fetus and stillbirth fetus of norfloxacin treated pregnant female rats from 5th-19th days of gestation.

Parameters / Groups	Total No. of sacrifice/ pregnant rats	Total No. of fetuses and Implantation site	No. of lives fetus		No. of resorped fetus		No. of dead fetus	
Control 0.5 ml DMSO	4	39 (8-12) 9.750 ± 1.137 a	39	100%	0	0%	0	0%
NFX 400mg/kg	4	28 (8-9) 7. 000 ± 2.403 c	15	53.57%	10	35.71%	3	10.72%
NFX 400mg/kg + CoQ10 200 mg/kg	4	37 (8-12) 9.250 ± 1.157 b	37	100%	0	0%	0	0%
NFX 400mg/kg + DHEA 25 mg/kg	4	36 (8-12) 9. 000 ±1.146 b	36	100%	0	0%	0	0%
NFX 400mg/kg + Combination CoQ10 200 mg/kg+ DHEA 25 mg/kg	4	38 (8-12) 9.500 ±1.153 b	38	100%	0	0%	0	0%

Small letters means significant differences between treatment at (P≤0.05)

Table (2): The effect of CoQ10 and DHEA and their combination on growth retardation rate represented by fetal body weight, fetal body length, fetal tail length, and placental weight, maternal weight gain of norfloxacin treated pregnant female rats from 5th - 19th day of gestation.

Parameters / Groups	Fetal body weight (F.WT) gm	Fetal body length (CRL) cm	Fetal tail length (F.TL) cm	Placental weight (P.WT) gm	Maternal weight gain (M.WT) gm
Control 0.5 ml DMSO	4.875± 0.11 a	5.19± 0.032 c	1.662± 0.043 d	0.605± 0.011 c	28.50±2.320 c
NFX 400mg/kg	2.466± 0.07 b	3.50± 0.123 a	1.283±0.060 a	0.490± 0.013 a	12.00±2.065 a
NFX 400mg/kg + CoQ10 200 mg/kg	4.453± 0.03 a	5.17± 0.041 b	1.542±0.040 a	0.509± 0.507 b	24.50±2.210 b
NFX 400mg/kg + DHEA 25 mg/kg	4.431± 0.02 a	5.13± 0.053 b	1.533±0.050 a	0.505± 0.015 b	23.30±2.320 ab
NFX 400mg/kg + Combination CoQ10 200 mg/kg+ DHEA 25 mg/kg	4.400± 0.06 a	5.10± 0.072 b	1.552± 0.042 a	0.603± 0.020 c	26.42±2.232 ab

Small letters means significant differences between treatment at ($P \leq 0.05$)

Table (3): Skull Bones of fetuses of various groups from pregnant female rats at 20th day of gestation. (+) ossified (-) non-ossified (% incidence of anomalies).

Parameters / Groups	Interparietal	Parietal	Nasal	Maxilla	Mandible
Control 0.5 ml DMSO	(+) Ossified 0 (0%)	(+) Ossified 0 (0%)	(+) Ossified 0 (0%)	(+) Ossified 0 (0%)	(+) Ossified 0 (0%)
NFX 400 mg/kg	- Un-ossified 7 (46.66%) * b	-Un-ossified 2 (13.33%)* a	-Un-ossified 2 (13.33%)* b	-Un-ossified 3(20.00%)* b	-Un-ossified 1(6.66%)* b
NFX 400 mg/kg + CoQ10 200 mg/kg	- Un-ossified 7(46.66%)* a	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b
NFX 400 mg/kg + DHEA 25 mg/kg	- Un-ossified 8 (53.33%)* a	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b	+ Ossified 0 (0%) b
NFX 400 mg/kg + Combination CoQ10 200 mg/kg + DHEA 25 mg/kg	+ Ossified 0 (0%)	+ Ossified 0 (0%)	+ Ossified 0 (0%)	+ Ossified 0 (0%)	+ Ossified 0 (0%)

Results are expressed as Mean ± SEM

Different letters represent significant difference at (P≤0.05)

*** Frequency of malformations, numbers of malformed fetuses, and % of malformations (No. of malformed fetuses/examined fetuses)**

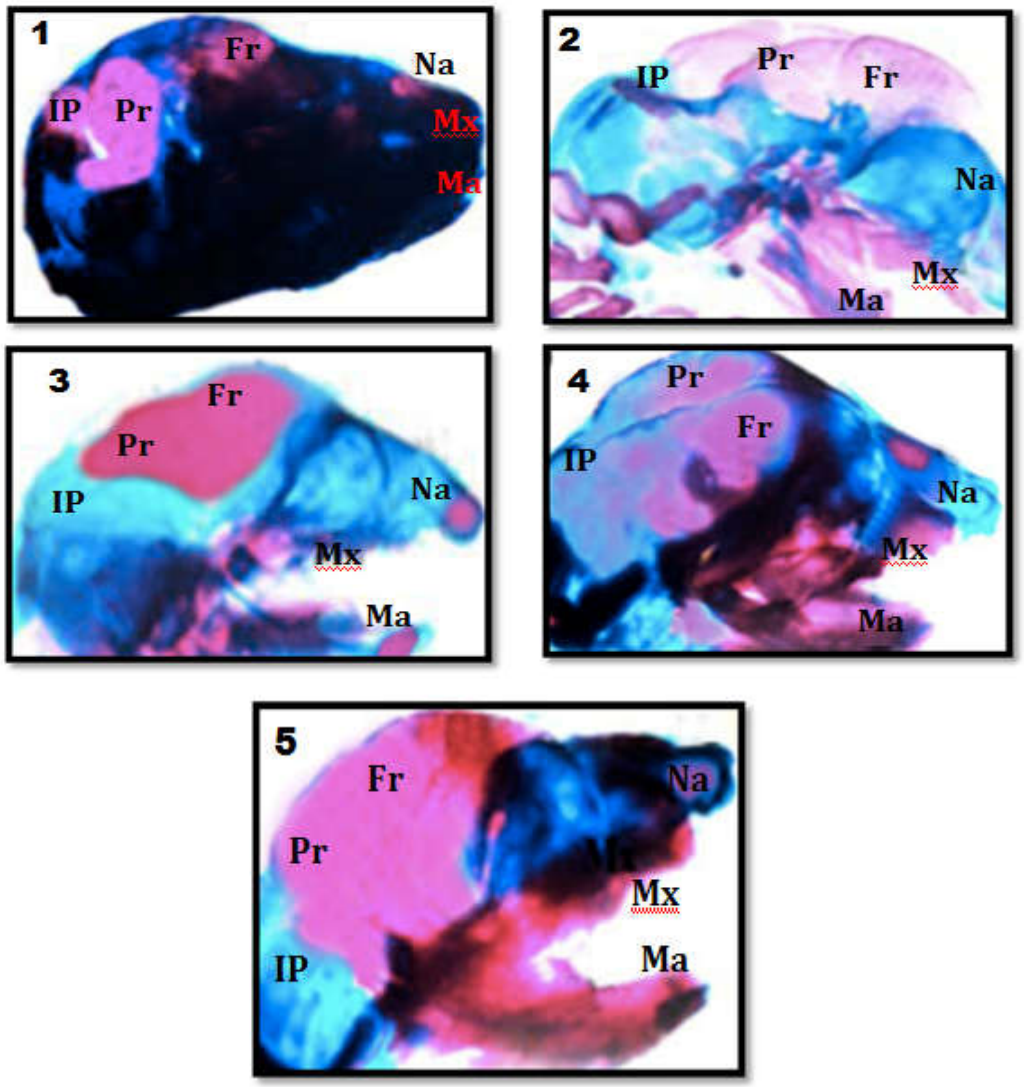

Figure (1): Photomicrographs of fetus cranial bones at 20th day of gestation (Alizarin red and Alcian blue stain). **(1) Control group:** Complete ossification and well-ossified of all cranial bones of fetus. **(2) Norfloxacin group:** Incomplete and lack of ossification of all cranial bones of the fetus compared to the control group. **(3) Norfloxacin + CoQ10 group:** Complete ossification and well-ossified of all cranial bones of fetus compared to the control after administration of 200 mg/kg of CoQ10, IP. **(4) Norfloxacin + DHEA group:** Complete ossification and well-ossified of all cranial bones of the fetus compared to the control after administration of 25 mg/kg of DHEA, IP. **(5) Norfloxacin + combination of CoQ10+DHEA group:** Complete ossification and well-ossified of all cranial bones of the fetus compared to the control after administration of combination 200 mg/kg of CoQ10+25 mg/kg of DHEA, IP. **IP = interparietal, Pr = parietal, Fr = frontal, Na = nasal, Mx = maxilla, and Ma = mandible**

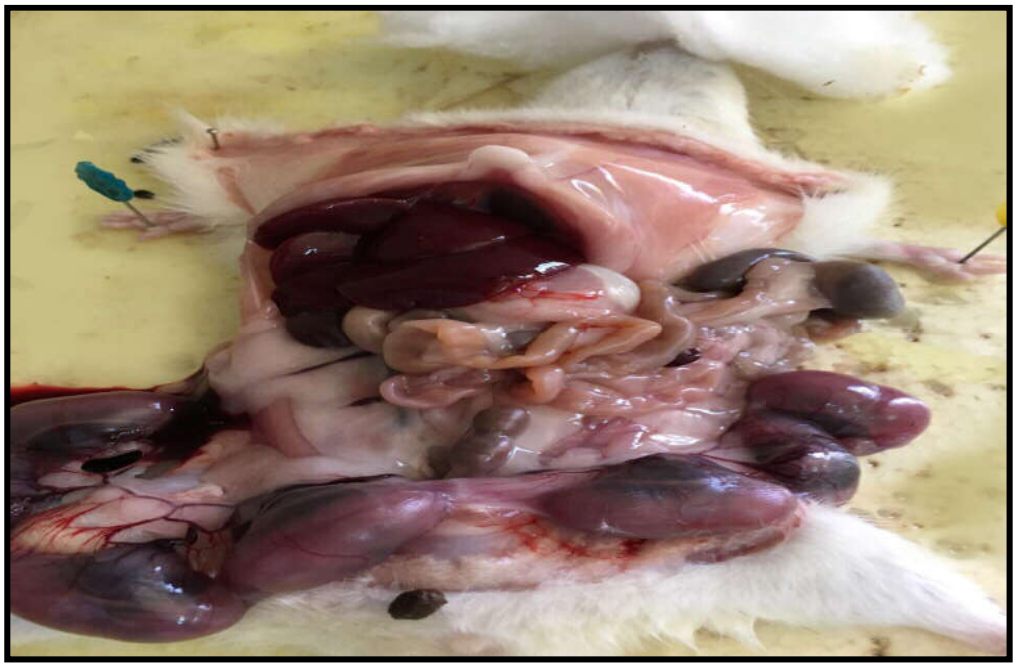

Figure (2): Uterine horns of control pregnant female rats treated daily with 0.5 ml DMSO on 20th days of gestation showing symmetrical distribution of fetuses in two uteri horns.

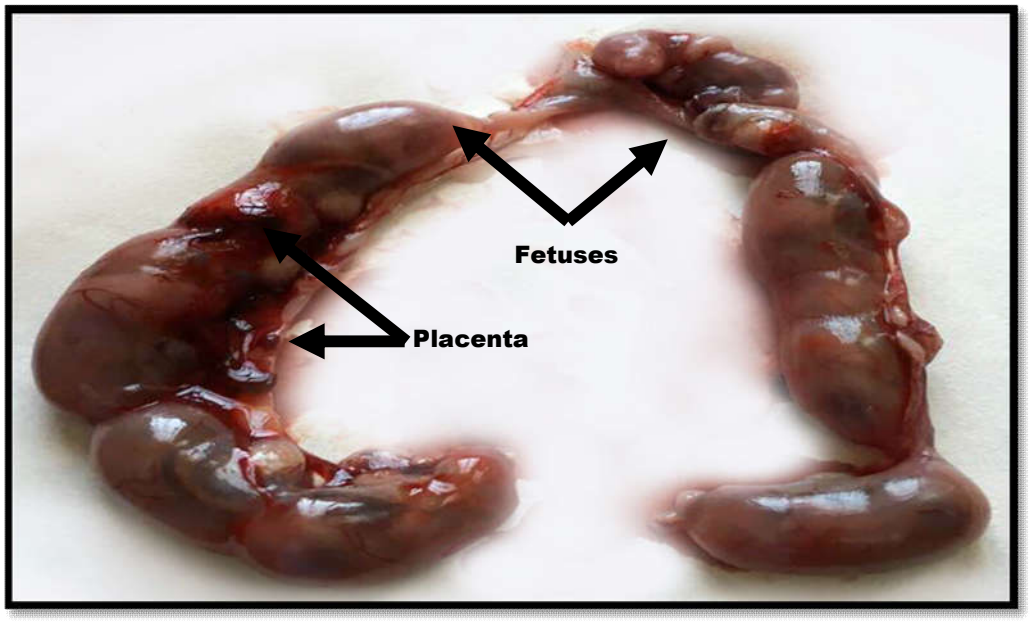

Figure (3): Uterine horns of norfloxacin treated pregnant female rats group on 20th days of gestation showing clearly visible resorped fetus with asymmetrical distribution of fetuses in two uteri horns.

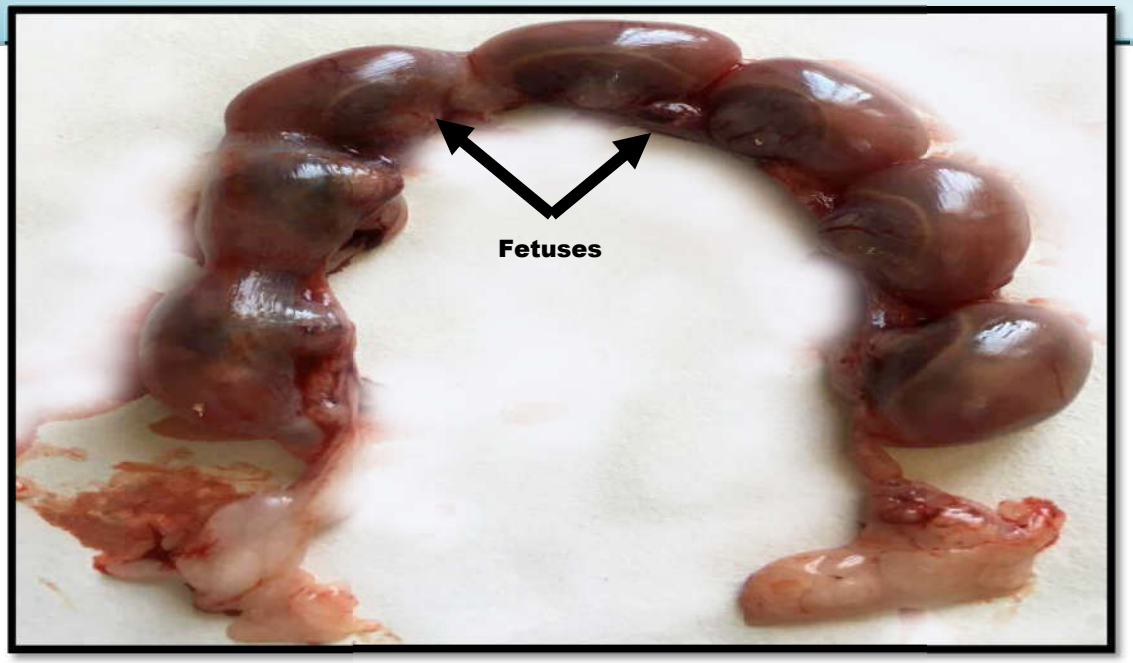

Figure (4): Uterine horns of norfloxacin group after administration with CoQ10 showing clearly improvements of uterine morphology with symmetrical distribution of fetuses in the two uteri horns.

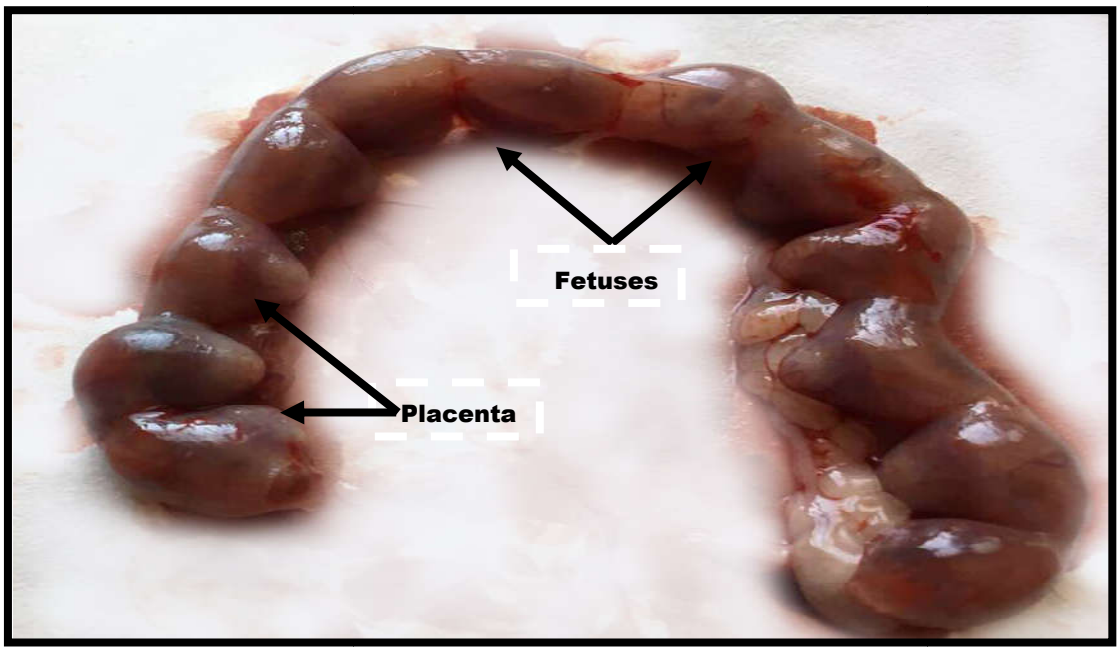

Figure (5): Uterine horns of norfloxacin group after administration with DHEA showing clearly improvements of uterine morphology with symmetrical distribution of fetuses in the two uteri horns.

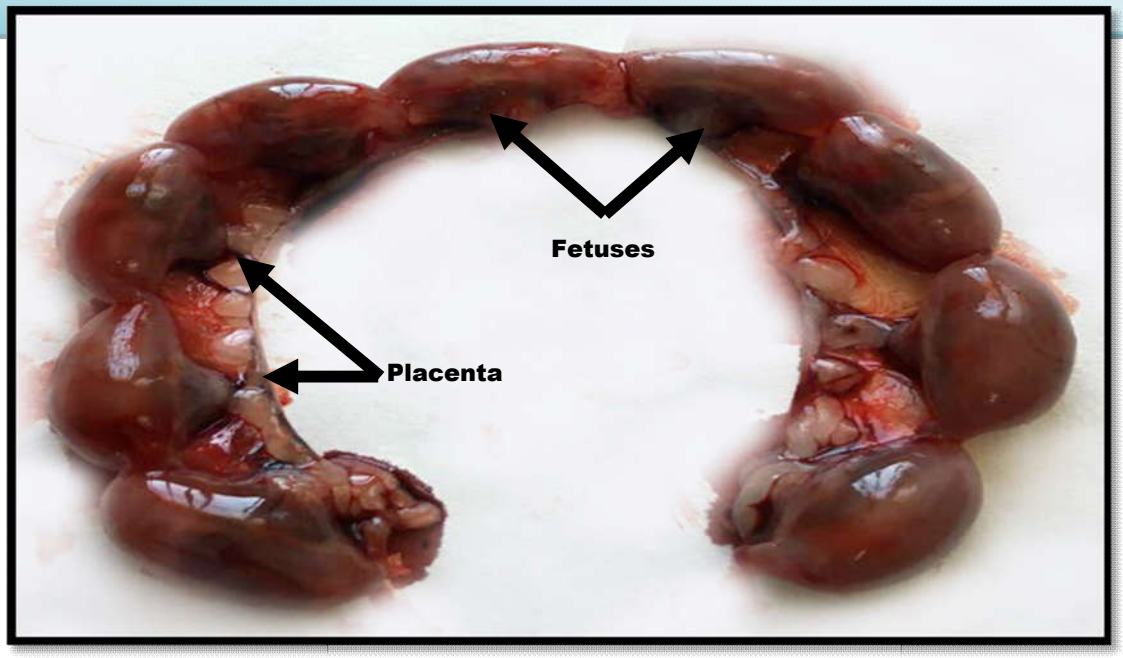

Figure (6): Uterine horns of norfloxacin group after administration with Combination of CoQ10 and DHEA showing clearly improvements of uterine morphology with symmetrical distribution of fetuses in the two uteri horns.

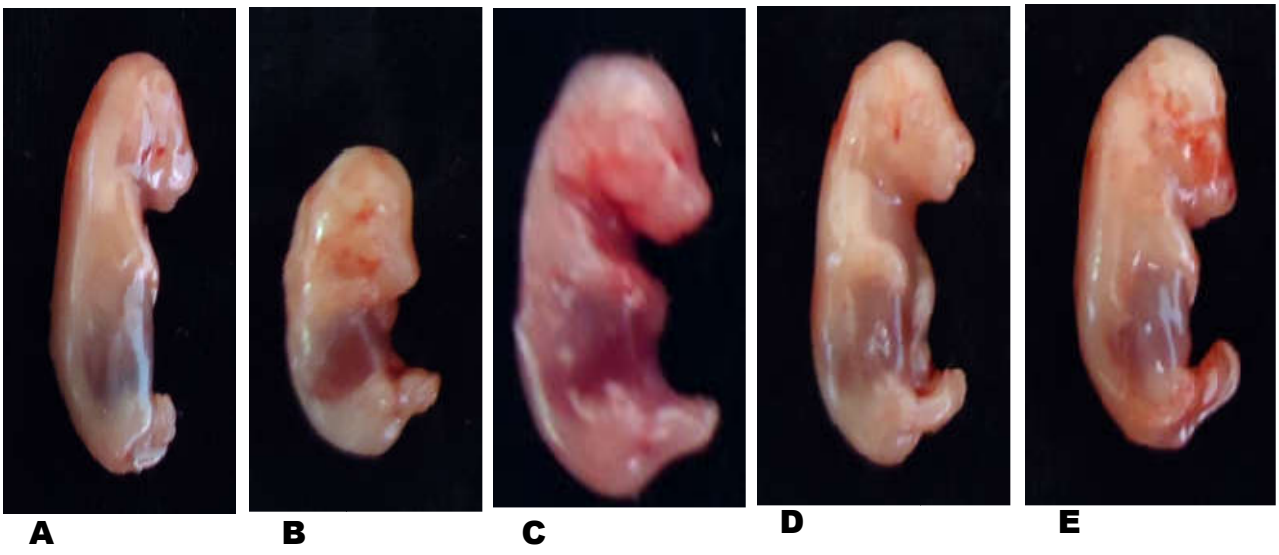

Figure (7): Different size of fetuses of different treated groups showing growth retardation (fetal body weight, fetal body length, and fetal tail length) from pregnant female rats at 20th day of gestation. **(A):** Control group treated with DMSO, **(B):** First treated group (NFX), **(C):** Second treated group (CoQ10), **(D):** Third treated group (DHEA), **(E):** Fourth treated group(CoQ10+DHEA).

DISCUSSION

In seems from the results of the present study that norfloxacin treated pregnant female rats during period of organogenesis from 5th -19th day of gestation caused significantly increased in resorped and death fetuses either early or late, and significantly decreased in number of viable fetuses compared to control group. These findings may be attributed to fluoroquinolones inhibitory effect on DNA gyrase, which is an enzyme necessary for negative super coiling twisting into double stranded DNA [22]. The inhibition of DNA synthesis induced by fluoroquinolones may be attributed to its ability to releasing oxygen free radicals [23]. It has been known that oxygen free radicals attack DNA causing mutations [24]. These results are agreed with those obtained by many researchers such as: [25] and [26], who studied embryotoxicity and teratogenicity of norfloxacin on pregnant female rats and their fetuses [27], who confirmed that fetotoxicity induced by norfloxacin may be attributed to interfering of used drug with placental transmission of amino acid leucin and magnesium from the dams to the fetus due to deficiency of these amino acid produced high incidence of fetal resorption rate, according to the negative relationship between parathyroid hormone secretion and magnesium deficiency due to relation hypoparathyroidism with the insufficiently low PTH levels. As well as, it's may be recognized to discontinued creation of placental progesterone when production of hormone switched from luteal to placental phase [28]. This results also may be attributed to inhibition of DNA transcription at late stage of rapidly divided fetal cells [29]. [30], mentioned that use of fluoroquinolone during first trimester of pregnancy caused failure in embryonic fixation which occurs after fertilization which led to early fetal death and increase fetal resorption. However, this study also showed decline in number of viable fetuses per pregnant dams. This results was reliable with the data reported by [31]. The decrease in the numbers of viable fetuses may be clarified on base of incomplete formation of placenta and degeneration of trophoblast and decidual cell, which play an important role in transmission of nutrients from the mother to the embryo [32].

It is also seems from the results that norfloxacin caused significantly decreased in fetal body weight, fetal body length (crown-rump length) and fetal tail length compared to control group. These results may be attributed to insufficiency of nutritional supply from the dam to the fetuses because female rats receiving drugs shows signs of soft stool or diarrhea due to imbalance in intestinal microflora, or may be due to trouble and drug interference in placental transmission in some minerals metabolism from the dams to the fetus such as magnesium and zinc which deficiency causes decreased in fetal growth retardation and increase in fetal

resorption and early embryonic death [33].The critical role of zinc in fetal skeletal system development may be attributed to stimulation of insulin like growth factor (IGF) which has essential function in fetal growth [34]. Also, zinc stimulate bone formation and mineralization, and increase bone alkaline phosphatase activity and DNA content [35]. [36], demonstrated that fetal body weight, fetal body length and fetal tail length significantly decreased after ciprofloxacin administration in pregnant female rats. Many earlier studies mentioned by [37] demonstrated decreased in fetal body weight, fetal crown-rump length and fetal tail length after orally administration of ofloxacin or levofloxacin to pregnant female rats. [38] showed that difloxacin decreased significantly fetal growth retardation after oral administration of 20 and 40 mg/kg of drug to pregnant female rats during period of organogenesis from 6^{th}- 15^{th} of gestation. However, other study stated that fluoride accumulation with repeated exposure to fluoroquinolone is related to the bone and cartilage damage [39]. The fluoroquinolone delayed developmental phase of epiphyseal growth with growth inhibition [40]. Furthermore, significantly decreased in maternal weight gain and placental weight compared to control group. These results may be attributed to decreased in diet intake and water consumption [41]. Many earlier studies reported that fluoroquinolones induced liver injury and hepatotoxicity through oxidative stress and generation of oxygen free radicals in the microsomal system with depression in endogenous antioxidant activity due to fluoride accumulation with repeated fluoroquinolone administration [42], due to drugs metabolism by cytochrome P450 and/or redox reaction. In addition to induced liver damage, drug also showed nephrotoxicity [43], cardiotoxicity [44], neurotoxicity [45], and placental toxicity. [46] showed that fluoroquinolone toxicity is associated with GABA-A receptor antagonism, resulting in decrease in the conductance of chloride ions. It has also been known that fluoroquinolone act as GABA antagonist in organism which blocks K channels connected to ATP [47]. As well as, it's related to NMDA receptor activation by eliminating Mg^{2+} block in ion channel. In contrast, lomefloxacin and norfloxacin blocked ATP sensitive K^{+} channels in pancreatic beta cells and increased insulin secretion [48].

It also seems from the results that norfloxacin treated pregnant female rats caused incomplete ossification of the skull bones compared to the control group. These results, as mentioned above, may be attributed to interference of norfloxacin with placental transmission of amino acid leucin and magnesium from dams to fetus due to deficiency of these amino acid produced high incidence of fetal resorption rate, according to negative correlation between parathyroid hormone secretion and magnesium deficiency results in hypoparathyroidism with insufficiently low PTH levels. Moreover, these relationship may be led to low bone turnover state, with

delayed calcium and phosphorus deposition into bone matrix and elevation levels of calcium and phosphorus, thus increase risk of metastatic calcification. The stability of this response suggests that impaired PTH secretion is an important factor causing hypocalcemia of magnesium deficiency, which produce fetal malformation either in growth or bone formation. [49] demonstrate that lack of ossification in the fetus may be attributed to oxidative stress by reducing mineralization of bone due to altered calcium metabolism or decreased levels of calcium and magnesium ion from bone tissue as well as altered calcitonin levels, thus induced changes in bone development and skeletal malformation. However, other study reported that fluoride accumulation with repeated fluoroquinolone administration is related to bone and cartilage damage. [50] reported that oxidative stress, ROS generation, lipid peroxidation and DNA damage of chondrocytes and collagen appears fluoroquinolones pathogenesis such as chrondrotoxicity, tendinopathy and tendon rupture induced by ciprofloxacin. These mechanism is associated with changes related with modification of metabolism and integrity of extracellular proteins. [51] also reported that oral administration of norfloxacin induced DNA damage in the liver and kidney of rats and targeted biological molecules such as DNA, lipids, proteins, and carbohydrates, which can result in embryo damage led to apoptosis and inhibiting DNA synthesis by interference of drugs with cell proliferation and differentiation and induction of cell death. In addition, reductions in uterine blood flow may be associated with the embryotoxicity.

In contrast, supplementation with CoQ10, DHEA and combination of CoQ10 and DHEA during period of organogenesis from 5^{th}-19^{th} days of gestation caused prevention and improved fetal skeletal malformation compared to norfloxacin treated group. These results may be attributed to a powerful anti-oxidant and anti-inflammatory properties against norfloxacin induced embryotoxicity and teratogenicity [52]. The present results in agreement with results obtained by [53] who they showed that CoQ10 administration to pregnant female rats caused prevent morphological and skeletal abnormalities induced by antidepressant drug. Also, who confirmed that antioxidant properties of CoQ10 make play a vital role in inhibiting bone resorption, cells differentiation, and protect osteoblast from oxidative damage induced by generated free radicals led to improved bone mineral density and stability. [54] stated that CoQ10 increased calcium absorption from gut, reduced urinary excretion of calcium, increased calcium deposition in bone, improved bone strength, and enhanced synthesis of bone collagen. [55] reported that a positive correlation between maternal CoQ10 and embryo development, in addition to balanced lipid metabolic alterations. [56] demonstrate the protective role of CoQ10 against oxidative stress, generation of ROS, and fetal cell death during pregnancy. For another

explanation, [57] demonstrate that stimulation of maternal immune system by administration of anti-oxidant and anti-inflammatory to the pregnant female rats can prevented and reduced fetal malformation of the drugs. However, many studies reported beneficial influences of maternal immune system on pregnancy outcome associated which reduced fetal teratogenicity through contribution of cytokines produced by immune cells [58], decrease of expression of TNF-α in fetal brain, modulation of fetal gene expression, inhibits pro-inflammatory cytokines synthesis, and enhances apoptosis by alteration cellular processes such as proliferation and differentiation [59]. [60] mentioned antioxidant and antimutagenic effect of vitamin C and β-carotene against oxidative stress and mutagenesis induced by Quinolones.

On the other hand, [61] showed that DHEA prevented loss in bone mineral density (BMD), decrease bone resorption, increase bone formation, increase production of bone cytokine and insulin like growth factor-1(IGF-1). However, [62] shows that IGF-1 or testosterone increased bone formation, while estrogen suppressed bone resorption. [63] showed a strong positive correlation between supplementation of DHEA and improved BMD, BMI, and IGF-1 levels, who explained that IGF-1 are growth promoting polypeptides play an important role in regulating osteoblastic and osteoclastic functions and maintaining bone mass, and are modulated by a groups of proteins like insulin-like growth factor binding proteins (IGFBPs) which is carried in a complex with IGFBP-3, which play a significant role in regulation of bone remodeling process in humans by enhancing anabolic effects of IGF-1 on bone. DHEA has anabolic effects and promotes APL activity and collagen I synthesis [64]. Furthermore, DHEA has a role in inhibition of interleukin 6 (IL-6) in skeletal muscle catabolism and stimulation of IGF-I-mediated mechanisms that underlie osteoanabolic events [65]. Many studies reported a helpful role of DHEA-S in bone strength. In another study, [66] reported that increased serum levels of DHEA-S is highly associated with reduced bone loss at femur neck and the lumbar spine. Furthermore, [67] reported that circulating DHEA have a direct effect on bone through a recognized DHEA receptor, or by conversion to androgens or estrogens within bone cells [68].

التأثير الوقائي لمرافق الانزيم Q-10 والاندروستيرون منزوع ثنائي الاوكسجين ومزجهما في التقليل من التأثير السمي وتشوهات الاجنة الناتج لعقار النورفلوكساسين في اناث الجرذان الحوامل

* باسم خميس كوتي الركابي ، ** محمد علي محمد الديوان ، *** علاء عبد الخالق حسين السواد

* قسم الانتاج الحيواني، كلية الزراعة,جامعة سومر، ذي قار، العراق

** فرع الفسلجة والادوية والكيمياء، كلية الطب البيطري,جامعة البصرة، البصرة، العراق

*** فرع التشريح والأنسجة والأجنة، كلية الطب البيطري,جامعة البصرة، البصرة، العراق

الـــخلاصـــة

صممت هذه الدراسة لتقييم الدور الوقائي لمرافق الانزيم Q-10 والاندروستيرون منزوع ثنائي الاوكسجين ومزجهما في ازالة التأثير السمي وتشوهات الاجنة الناتج من التعرض لعقار النورفلوكساسين في اناث الجرذان الحوامل. اجريت هذه الدراسة على ٢٠ من اناث الجرذان الحوامل مقسمة الى خمس مجاميع متساوية، اربعه اناث في كل مجموعة وكالآتي: مجموعة السيطرة: ٤ اناث حوامل جرعت بـ 0.5 مل من مادة ثنائي اوكسيد سلفات المثيل لكل حيوان في اليوم الواحد عن طريق الفم باستخدام الانبوب المعدي من اليوم الخامس الى اليوم التاسع عشر من الحمل. مجموعة المعاملة الأولى: ٤ اناث حوامل جرعت بعقار النورفلوكساسين بتركيز 400 mg/kg لكل حيوان في اليوم الواحد عن طريق الفم باستخدام الانبوب المعدي. مجموعة المعاملة الثانية: ٤ اناث حوامل جرعت بعقار النورفلوكساسين بتركيز 400 mg/kg لكل حيوان في اليوم الواحد عن طريق الفم باستخدام الانبوب المعدي ثم بعد مرور ساعة حقنت بعقار مرافق الانزيم Q-10 بتركيز 200 mg/kg داخل التجويف البريتوني. مجموعة المعاملة الثالثة: ٤ اناث حوامل جرعت بعقار النورفلوكساسين بتركيز 400 mg/kg لكل حيوان في اليوم الواحد عن طريق الفم باستخدام الانبوب المعدي ثم بعد مرور ساعة حقنت بعقار الاندروستيرون منزوع ثنائي الاوكسجين بتركيز25 mg/kg داخل التجويف البريتوني. مجموعة المعاملة الرابعة: ٤ اناث حوامل جرعت بعقار النورفلوكساسين بتركيز 400 mg/kg لكل حيوان في اليوم الواحد عن طريق الفم باستخدام الانبوب المعدي ثم بعد مرور ساعة حقنت بمزيج من عقار مرافق الانزيم Q-10 بتركيز 200 mg/kg والاندروستيرون منزوع ثنائي الاوكسجين بتركيز 25 mg/kg داخل التجويف البريتوني ولنفس الفترة. قتلت الاناث في اليوم ٢٠ من الحمل وتم استخراج الاجنة من الرحم في كل مجموعة لمعرفة مدى حدوث تشوهات شكلية وهيكلية باستخدام تقنية الصبغات الخاصة المزدوجة (صبغة الاليزارين الحمراء والاليشين الزرقاء) لغرض تصبيغ العظم والغضروف.

اظهرت نتائج الدراسة الحالية بان التعرض الى عقار النورفلوكساسين في اناث الجرذان الحوامل خلال فترة الحمل من اليوم الخامس الى اليوم التاسع عشر يسبب زيادة في معدل وفيات الاجنة (الارتشاف الجنيني والأجنة الميتة)، ونقصان في معدل تخلف النمو(اوزان الأجنة ، اطوال الأجنة ، ومعدل طول الذيل)، اوزان الامهات والمشائم، اضافة الى ذلك حدوث تشوهات هيكلية في عظام الجمجمة(عظام القحف) مقارنة بمجموعة السيطرة. نستنتج من هذه الدراسة بان مرافق الانزيم Q-10 والاندروستيرون منزوع ثنائي الاوكسجين ومزجهما يلعب دوراً وقائياً مهماً في منع ومعالجة التشوهات الشكلية والهيكلية اثر التعرض الى عقار النورفلوكساسين في اجنة الجرذان. وبالتالي فان مرافق الانزيم Q-10 والاندروستيرون منزوع ثنائي الاوكسجين يعتبران من مضادات الاكسدة القوية للحيلولة دون حدوث تسمم وتشوهات في الاجنة نتيجة التعرض الى اجهاد تأكسدي تم توليده من قبل عقار نورفلوكساسين.

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