Basrah Journal of Veterinary Research,Vol.17, No.3,2018
Proceeding of 6th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

AMELIORATIVE EFFECT OF COQ10, DHEA AND THEIR COMBINATION ON EMBRYOTOXICITY AND TERATOGENICITY INDUCED BY NORFLOXACIN IN PREGNANT FEMALE RATS

Bassim K. Kuoti Al-Rekabi *; Mohammed A. Al-Diwan ** ; Alaa A. Sawad ***

* Department of Animal Production, College of Agriculture, University of Sumer, 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.

Key words: CoQ10, DHEA, Maternal Toxicity, FGR, Norfloxacin

Corresponding authors E. Mail: kh_bassim@yahoo.com

ABSTRACT

This study was designed to evaluate the ameliorating role of CoQ10 and DHEA and their combination on norfloxacin induced maternal toxicity and fetal growth retardation in pregnant female rats and their fetuses. Twenty pregnant rats were divided equally into 5 groups, 4 animals per each group as following: Control group (G1): 4 normal pregnant female received 0.5ml/animal/day DMSO by using gastric gavages from 5^{th} - 19^{th} day of gestation. First treated group (T1): 4 normal pregnant female received 400 mg/kg norfloxacin daily by gastric gavages as a single dose. Second treated group (T2): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hour later 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 1hour later injected daily with DHEA 25 mg/kg IP. Fourth treated group (T4): 4 normal pregnant female received orally 400 mg/kg norfloxacin once daily and after 1hour later injected daily with combination of CoQ10 200 mg/kg and DHEA 25 mg/kg IP for the same period. All dams were sacrificed at 20^{th} day of gestation and their fetuses were collected and subjected to the morphological examinations. The obtained results showed that exposure to norfloxacin in pregnant female rats during gestational period from 5^{th} -19^{th} day demonstrate clearly increased in resorbed and stillbirth fetuses (dead fetuses at birth), and caused a significant decreased in fetal body weight, fetal body length, fetal tail length, maternal weight gain and placental weight. It has

been concluded that CoQ10 and DHEA prevented and treated morphological anomalies in rat fetuses. Therefore, CoQ10 and DHEA are potential therapeutic antioxidant agents against fetotoxicity 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]. It 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 agent that is able to cross placental barrier and enter fetal circulation. It acts by inhibition of bacterial DNA replication by inhibition of bacterial DNA gyrase and topoisomerase II enzyme [5]. Therefore, every agent given during pregnancy 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 development in rat and mice is from 6-15 days of their gestation [7]. A birth defect or 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 occur 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, and plays an essential role in mitochondrial energy production through redox activity in the electron transport chain, transporting electrons between enzymes. Thus, it plays an essential role in cellular bioenergetics and membrane stabilizer and production of ATP in oxidative respiration process [12]. CoQ10 has anti-inflammatory properties decreasing production of pro-inflammatory cytokines such as

interleukin (IL) and tumor necrosis factor (TNF-α) [13]. Dehydroepiandrosterone (DHEA) is one of the most abundant endogenous circulating steroid hormones 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 has 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 occur in mitochondria and are resulting from the action of cytochrome P450 [17]. This study aimed to evaluate the ameliorating role of CoQ10 and DHEA and their combination on maternal toxicity and fetal growth retardation in pregnant female rats exposed to norfloxacin.

MATERIALS 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 after dissolving 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 0.5ml/animal/day DMSO by using gastric gavages 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 1hour 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 1hour injected daily with DHEA 25 mg/kg IP. 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 fetuses and resorption sites were observed. Fetal growth retardation represented by fetal body weight, fetal body length or crown rump 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 (resorbed or dead fetuses), maternal weight gain, placental weight, living fetuses were evaluated.

Statistical Analysis

In this study, one way ANOVA analysis and LSD tests are used according to Statistical Package for Social Sciences (SPSS, version 13) program. The data were expressed as Mean ± standard deviation (Mean ± SD). Least significant difference test (LSD) was used to test the difference between means (groups); $P \leq 0.05$ was considered significant (SPSS, 2001).

RESULTS

Maternal rats toxicity

Maternal weight gain and placental weight changes

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

Effect of norfloxacin (NFX) on developing fetuses

Growth retardation rate

The results on table (2) and figure (1) also pointed out that the pregnant female rats received norfloxacin showed significant decrease (P≤ 0.05) in the fetal body weight, fetal body length and fetal tail length compared to control group. Whereas, groups treated with CoQ10, DHEA and combination of CoQ10 and DHEA showed a significant increase (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 (1) 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.

Total prenatal mortality rate

The results in table (1) illustrate a clearly increased 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 clearly reduced in resorbed and dead fetuses on day 20th of gestation compared to groups treated with norfloxacin, but they still less compared to the control value. It is also observed from table (1) that combination of CoQ10 and DHEA caused a highly decreased in resorbed and stillbirth and almost returns to its normal levels compared with control value.

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 resorbed fetus		No. of dead fetus	
Control 0.5 ml DMSO	4	39 (8-12) 9.750 ± 1.137	39	100%	0	0%	0	0%
NFX 400mg/kg	4	28 (8-9) 7. 000 ± 2.403	15	53.57%	10	35.71%	3	10.72%
NFX 400mg/kg + CoQ10 200 mg/kg	4	37 (8-12) 9.250 ± 1.157	37	100%	0	0%	0	0%
NFX 400mg/kg + DHEA 25 mg/kg	4	36 (8-12) 9. 000 ±1.146	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	38	100%	0	0%	0	0%

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

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

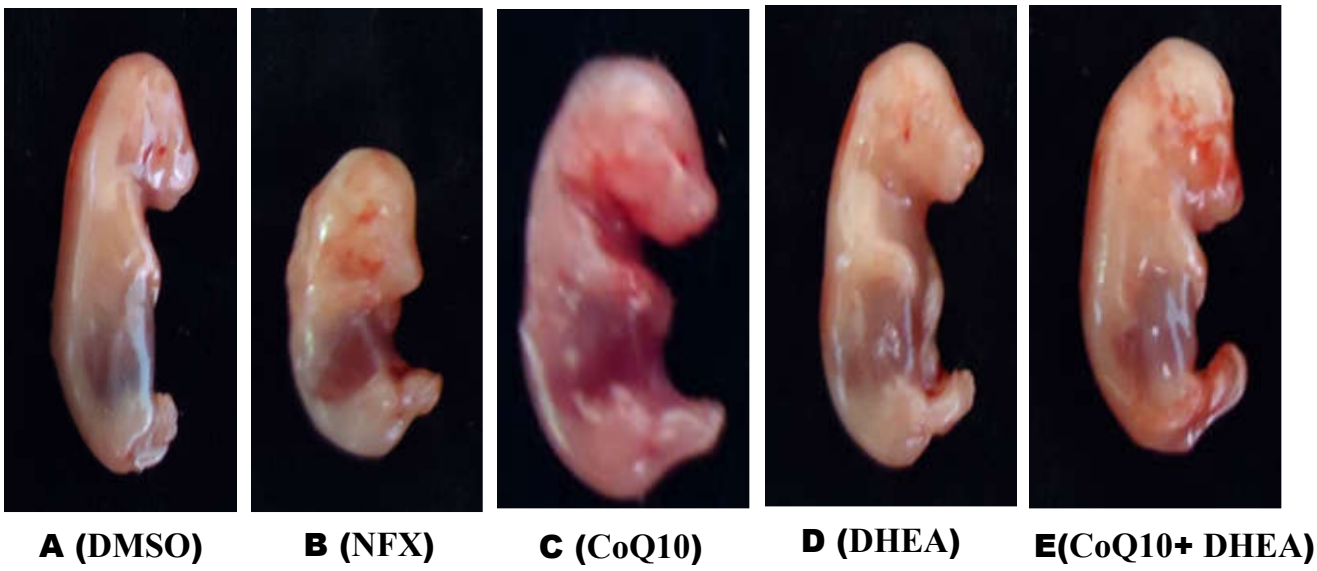

Figure (1): Different size of fetuses of different treated groups showing growth retardation (fetal body weight, fetal body length, and fetal tail length) from pregnant female rats at 20th day of gestation. **(A):** Control group treated with DMSO, **(B):** First treated group (NFX), **(C):** Second 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 5^{th} -19^{th} 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 [21]. The inhibition of DNA synthesis induced by fluoroquinolones may be attributed to its ability to releasing oxygen free radicals [22]. It has been known that oxygen free radicals attack DNA causing mutations [23]. These results are agreed with those obtained by many researchers such as: [24] and [25], who studied embryotoxicity and teratogenicity of norfloxacin on pregnant female rats and their fetuses [26], 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 may be recognized to discontinue creation of placental progesterone when production of hormone switched from luteal to placental phase [27]. These results also may be attributed to inhibition of DNA transcription at late stage of rapidly divided fetal cells [28]. [29], 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 result was reliable with the data reported by [30]. 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 [31].

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 result 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 [32].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 [33]. Also, zinc stimulate bone formation and mineralization, and increase bone alkaline phosphatase activity and DNA content [34]. [35], 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 [36] 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. [37] 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 [38]. The fluoroquinolone delayed developmental phase of epiphyseal growth with growth inhibition [39]. In addition, significantly decreased in maternal weight gain and placental weight compared to control group. These result may be attributed to decrease in diet intake and water consumption [40]. 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 [41], due to drugs metabolism by cytochrome P450 and/or redox reaction. In addition to induced liver damage, drug also showed nephrotoxicity [42], cardiotoxicity [43], neurotoxicity [44], and placental toxicity. [45] 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 [46]. 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 [47].

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 [48]. The present results are in agreement with results obtained by [49] who they showed that CoQ10 administration to pregnant female rats

caused prevent morphological and skeletal abnormalities induced by antidepressant drug. Also, it is confirmed that antioxidant properties of CoQ10 might 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. [50] 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. [51] reported that a positive correlation between maternal CoQ10 and embryo development, in addition to balanced lipid metabolic alterations. [52] demonstrate the protective role of CoQ10 against oxidative stress, generation of ROS, and fetal cell death during pregnancy. For another explanation, [53] 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 [54], 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 [55]. [56] mentioned antioxidant and antimutagenic effect of vitamin C and β-carotene against oxidative stress and mutagenesis induced by Quinolones.

On the other hand, [57] 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, [58] shows that IGF-1 or testosterone increased bone formation, while estrogen suppressed bone resorption. [59] 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 [60]. 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 [61]. Many studies reported a helpful role of DHEA-S in bone strength. In another study, [62] reported that increased serum levels of DHEA-S is highly associated with reduced bone loss at femur neck and the lumbar

spine. Furthermore, [63] 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 [64].

REFRENCES

1. **Wells, P.G., McCallum, G.P. and Chen, C.S. (2009b).** Oxidative stress in developmental origins of disease: tera togenesis, neurodevelopmental deficits and cancer. Toxicol Sci., 108:4–18.
2. **Thompson, J., Doi, T., Power, E., Balasubramanian, I., Puri, P. and Bannigan, J. (2010).** Evidence against a direct role for oxidative stress in cadmiuminduced axial malformation in the chick embryo. Toxicol Appl Pharmacol., 243:390-398.
3. **Robertson, D.G.; Epling, G.A.; Kelly, J.S.; Bailey, D.L. and Song, B. (1991).** Mechanistic studies on the phototoxic potential of PD 117596, a quinolone antibacterial compound, Toxicol. Appl. Pharmacol., 111: 221-232.
4. **Saracolglu, A.; Temel, H.E.; Ergun, B. and Colak, O. (2009).** oxidative stress mediated myocardiotoxicity of ciprofloxacin and ofloxacin in juvenile rats. Drug chemical toxicil., 32: 238-242.
5. **Elsea, S.H.; Mcguirk, P.R.; Gootz, T.D.; Moynihan, M. and Osheroff, N. (1993).** Drugs features that contribute to the activity of quinolone against mammalian topoisomerase II and cultured cells. Antimicrob. Agents Chemother. 37(1.0): 2179-2186.
6. **Schlegel, P.N., Chang, T.S. and Marshall, F.F. (1991).** Antibiotics: potential hazards to male fertility. Fertil Steri, , vol. 55, p. 235‑242.
7. **Somer, G.F. (1962).** Thalidomide and Congenital Abnormalities. The Lancet, 1: 912-913. S. Abo-Kora et l.152http://dx.doi.org/10.1016/S0140-6736(62) 91943-8.
8. **Moore, K. (1988).** The Developing Human. 4th Edition, WB Saunder, Philadelphia.
9. **Briggs, G.G., Freeman, R.K. and Yaffe, S.J. (2005).** Drugs in pregnancy and Lactation , 7th ed .Philadelphia : Lippincott Williams and Wilkins.
10. **Ali, S.A.; Faddah, L.; Abdel-Baky, A. and Bayoumi, A. (2010).** Protective effect of L-Carnitine and coenzyme Q10 on CCl_4-induced liver injury in Rats. Sci. Pharm. 78(4): 881-896.

11. **Tran, M.T.; Mitchell, T.M.; Kennedy, D.T.and Giles, J.T. (2001).** Role of coenzyme Q10 in chronic heart failure, angina, and hypertension. Pharmacotherapy., 21(7):797-806.

12. **López, L., Quinzii, C., Area, E., Naini, A., Rahman, S., Schuelke, M., Salviati, L., Dimauro, S . and Hirano, M. (2010).** Treatment of CoQ10 deficient, fibroblasts with Ubiquinone, CoQ analogs, and vitamin C: time- and compound-dependent effects. PLOS One, 5(7): 897-903.

13. **Sayed-Saleh, A.B.; Shahin, M.I. and Kelada, N.A. (2017).** Hepatoprotective effect of taurine and coenzyme Q10 and their combination against acrylamide-induced oxidative stress in rats. Tropical Journal of Pharmaceutical Research August., 16 (8): 1849-1855.

14. **Prough, R.A.; Clark, B.J. and Klinge, C.M. (2016).** Novel mechanisms for DHEA action. J Mol Endocrinol., 56(1): 139-155.

15. **Kim, B.M.; Yim, S.H.; Jeong, S.J.; Choi, Y.S.; Nam, Y.S.; Jeong, J.H.; Yun, S.W.; Do, J.H.; Lim, H.M. and Park, E.S. (2009).** Pro-oxidantive effect of dehydroepiandrosterone on indomethacin induced acute gastritis in rats. Biomolecules and Therapeutics., 17(1): 57-61.

16. **Du, M.C.; Khalil, W. and Sriram, S. (2017).** Administration of Dehydroepiandrosterone Suppresses Experimental Allergic Encephalo- myelitis in SJL/J Mice. J.immunology .,167:7094-7101.

17. **Miller, W.L. and Auchus, R.J. (2011).** The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. Endocr Rev., 32(1): 81–151. doi: 10.1210/er.2010-0013.

18. **Marcondes, F.K., Bianchi, F.J. and Tanno, A.P. (2002).** Determination of the estrus cycle phases of the rats: Some helpful considerations. Brazilizn J. Biol. 62: 609-614.

19. **Macintyre, D.J., Chang, H.H. and Kaufman, M.H., (1995).** Teratogenic effects of amniotic sac puncture: a mouse model. Journal of Anatomy., 186: 527-539.

20. **Chang, H.H., Schwartz, Z. and Kaufman, M.H. (1996).** Limb and other Post Cranial Skeletal Defects induced by Amniotic sac puncture in mouse. J Anat. 189: 37-49.

21. **Vancutsem, P.M., Babish, J.G. and Schwark, W.S. (1990).** The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet. 80(2): 173-86.

22. **Gürbay, A., Gonthier, B., Signorini-Allibe, N., Barret , L., Favier, A. and Hincal, F. (2006).** Ciprofloxacin-induced DNA damage in primary culture of rat astrocytes and protection by vitamin E. Neurotoxicol., 27: 6-10.

23. **Arriaga-Alba, M., Rivera-Sánchez, R., Parra-Cervantes, G., Barron Moreno, F., Flores-Paz, R. and Elbia García-Jiménez, E. (2000).** Antimutagenesis of b-carotene to mutations induced by quinolone on Salmonella typhimurium. Arch Med Res., 31:156–161.

24. **Aboubakr, M., Elbadawy, M., Soliman, A. and El-Hewaity, M. (2014).** Embryotoxic and Teratogenic Effects of Norfloxacin in Pregnant Female Albino Rats. Advances in Pharmacological Sciences., 6(2): 1-6.

25. **El-Komy, A., El-Meleh, A., El-zoghby, R. and Salem, A. (2017).** Effect of Norfloxacin on fetal development in pregnant female albino rats. WJPPS., 6 (3):46-59.

26. **Tuchmann-Duplessis, H. (1975).** Drug effect on the fetus. ADIS press, New York, USA.

27. **Hummler, H., Richter, W.F. and Hendrickx, A.G. (1993).** Developmental toxicity of fleroxacin and comparative pharmacokinetics of four fluoroquinolones in cynomolgus macaque (Macacafascicularis). Tox. App. Pharm., 122 (l): 34-45.

28. **Al-Snaffi, A. E. and Shafik, N.A. (1997).** Embryotoxicity of Norfloxacin in mice. The Medical Journal of Tikrit University. 3: 200-203.

29. **Cono, J., Cragan, J.D., Jamieson, D.J. and Rasmussen, S.A. (2006).** Prophylaxis and treatment of pregnant women for emerging infections and bioterrorism emergencies. Emerg Infect Dis. Nov.12(11):1631-7.

30. **Eteng, M.U., Ukpanukpong, R.U., Abolaji, A.O., Eyong, E.U. and Eteng, E. (2008).** Biochemical and Histological Alteration and Effect of Perfloxacin on Wistar Rats Reproductive Function. Aust. J. Bas. App. Sci., 2(3): 475-480.

31. **Kurebe, M., Asaoka, H. and Moriguchi, M. (1984).** Toxicological studies on a new cephamycin, MT-141. IX. Its teratogenicity test in rats and rabbits. Japanese Journal ofAntibiotics., 37(6):1186–1210.

32. **Clemens, G.R. and Hartnagel, R.E. (1985).** A teratology (Segment II) study in the rat with BAY Vp 2674. Unpublished Report No. 53 from the Toxicology Department, Central Research Services, Miles.

33. **Hanna, L.A., Clegg, M.S., Ellis-Hutchings, R.G., Niles, B.J. and Keen, C.L. (2010).** The influence of gestational Zinc deficiency on the fetal insulin-like growth factor axis in the rat. Experimental Biology and Medicine; 235 (2): 206-214.

34. **Yamaguchi, M. (1998).** Role of Zinc in bone formation and bone resorption. Journal of Trace Elements in Experimental Medicine; 11: 119-135.

35. **Siddiqui, M.A. and Naqvi, S.N.H. (2010).** Evaluation of the teratogenic potentials of ciprofloxacin in albino rat J. Morphol. Sci., 27(1): 14-18.

36. **Watanabe, T., Fujikawa, K., Harada, S., Ohura, K., Sasaki, T. and Takayama, S. (1992).** Reproductive toxicity of the new quinolone antibacterial agent levofloxacin in rats and rabbits. Arzneimittel- Forschung., 42(3): 374–377.

37. **El-komy, A., Aboubakr, M. and Medhat, N. (2016).** Some teratological effects of difloxacin in rats. BVMJ., 30(1): 266-271.

38. **Arora, N.K. (1994).** Are Fluoroquinolones safe in children?. India J Pediatr. 61(6): 601-603.

39. **Stahlmann, R., (2003).** Children as Special Population on Risk – Quinolones as example for xenobiotics exhibiting skeletal toxicity. Arch toxicol., 77(1): 7 - 11.

40. **Kim, J.G., Yun, H.I., Shin, H.C., Han, S.S. and Chung, M.K. (2000).** Embryo lethality and teratogenicity of a new fluoroquinolones antibacterial DW-116 in rats. Arch. Toxical. 74: 120-124.

41. **Zimpfer, A., Propst, A., Mikuz, G., Vogel, W., Terracciano, L. and Stadlmann, S. (2004).** Ciprofloxacin-induced acute liver injury: case report and review of literature.Virchows Arch., 444(1):87-89.

42. **Al-shawi, N.N. (2012).** Possible histological changes induced by therapeutic doses of ciprofloxacin in liver and kidney of Juvenile rats pharmacologica. 3(9):42-47.

43. **Shin, H.C., Kirn, J.C., Chung, M.K, Jung, Y.H., Kirn, J.S., Lee, M.K. and Gordon, L.A. (2003).** Fetal and maternal tissue distribution of the new fluoroquinolone DW-116 in pregnant rats. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 136 (1): 95-102.

44. Akahane, K., Kato, M. and Takayama, S. (1993). Involvement of inhibitory and excitatory neurotransmittors in levofloxacin and ciprofloxacin induced convulsions. Antimicrob. Agents C58882 he mother. 37(9): 1764-1776.

45. **Akahene, K.M., Sekiguchi, T., Une, N. and Osadas, Y. (1989).** Structure-epileptogenicity relationship of quinolones with special reference to their interaction with gamma-aminobutyric acid receptor sites. Antimicrob Agents Chemother., 33: 1704-1708.

46. **Becker, B., Antoine, M.H., Nguyen, Q.A., Rigo, B., Cosgrove, K.E., Barnes, P.D., Dunne, M.J., Pirotte, B. and Lebrun, P. (2001).** Synthesis and characterization of a quinolinonic compound activating ATP-sensitive K+ channels in endocrine and smooth muscle tissues. Br J Pharmacol., 134: 375-385.

47. **Saraya, A., Yokokura, M., Goni, T. and Seino, S. (2004).** Effect of fluoroquinolones on insulin secretion on B- cell ATP-sensitive K^+ channels. Eur J Pharmacol., 497:111-117.

48. **Winn, L.M. and Wells, P.G. (1999).** Maternal administration of superoxide dismutase and catalase in phenytoin teratogenicity. Free. Radic. Biol. and Med., 26: 266-274.

49. **Abu Gabal, H. and Al Shabanat, F. (2012).** The role of Coenzyme Ubquinone CoQ10 in modulating the changes induced by the antidepressant Venlafaxine in albino rats fetuses. Egyptian Journal of Hospital Medicine., 46: 64 – 82.

50. **Moon, H. J.; Ko, W. K.; Han, S.W.; Kim, D.S.; Hwang, Y. S.; Park, H. K. and Kwon, I.K. (2013).** Antioxidants, like coenzyme Q10, selenite, and curcumin, inhibited osteoclast differentiation by suppressing reactive oxygen species generation. Biochem. Biophys. Res. Commun., 418, 247-253.

51. **Haruna, M., Matsuzaki, M., Tanizaki, T., Sekine, K., Ota, E. and Murashima, S. (2010).** Increased serum coenzyme Q10 during pregnancy correlates to birth weight. Biofactors, 36 (4), 312–318.

52. Quinzii, C., López, L., Gilkerson, R., Dorado, B., Coku, J., Lagier-Tourenne, C., Schuelke, M., Salviati, L., Carrozzo, R., Santorelli, F., Rahman, S., Tazir, M., Koenig, M., Di Mauro, S. and Hirano, M. (2010). Reactive oxygen species, oxidative stress, and cell death correlate with level of CoQ10 deficiency. FASEB. J., 24(10):3733-43.

53. **Holladay, S.D., Sharova, L.V., Punareewattana, K., Hrubec, T.C., Gogal, R.M., Prater, M.R. and Sharov, A.A. (2002).** Maternal immune stimulation in mice decreases fetal malformations caused by teratogens. Int. Immunopharmaco., 2: 25-332.

54. **Nomura, T., Hata, S. and Kusafuka, T. (1990).** Suppression of developmental anomalies by maternal macrophages in mice. J. Exp. Med., 172(5): 1325-30.

55. **Ivnitsky, I., Torchinsky, A., Savion, S., Shepshelovich, J., Orenstein, H., Toder, V. and Fein, A. (2001).** TGF beta2 in embryos with inborn anomalies: effect of maternal immunopotentiation. Am. J. Reprod Immunol., 45(1): 41-51.

56. **Arriaga-Alba, M., Rivera-Sánchez, R., Flores-Paz, R., Torres-Ramos, Y.D., Olivares-Corichi, I.M. and Hicks, J.J. (2008).** Antimutagenic effects of vitamin C Against oxidative changes induced by Quinolones. Food Technol. Biotechnol. 46 (1) 38-43.

57. **Jankowski, C.M., Gozansky, W.S. and Schwartz, R.S. (2006).** Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial. J Clin Endocrinol Metab., 91:2986-93.

58. **Prestwood, K.M., Kenny, A.M., Kleppinger, A. and Kulldorff, M. (2003).** Ultralow-dose micronized 17beta-estradiol and bone density and bone metabolism in older women: a randomized controlled trial. JAMA., 290:1042-1048.

59. Park, S.G., Hwang, S., Kim, J.S., Park, K.C., Kwon, Y. and Kim, K.C. (2017). The Association between Dehydroepiandrosterone Sulfate (DHEA-S) and Bone Mineral Density in Korean Men and Women. J Bone Metab., 24:31-36.

60. **Gordon, C.M., Glowacki, J. and LeBoff, M.S. (1999).** DHEA and the skeleton (through the ages). Endocrine., 11:1-11.

61. **Morales, A.J., Nolan, J.J. and Nelson, J.C. (1994).** Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. J Clin Endocrinol Metab., 78:1360-7.

62. **Ghebre, M.A., Hart, D.J. and Hakim, A.J. (2011).** Association between DHEAS and bone loss in postmenopausal women: a 15- year longitudinal population-based study. Calcif Tissue Int., 89:295-302.

63. **Wang, L., Wang, Y.D., Wang, W.J., Zhu, Y. and Li, D.J. (2007).** Dehydroepiandrosterone improves murine osteoblast growth and bone tissue morphometry via mitogen-activated protein kinase signaling pathway independent of either androgen receptor or estrogen receptor. J Mol Endocrinol., 38: 467-79.

64. **Labrie, F., Luu-The, V., Labrie, C. and Simard, J. (2001).** DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol., 22:185-212.