

Evaluation of the genetic effects of meiacet alone or in combination with carnitine on pregnant female mice and embryos

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Abstract : Pivalate-generating prod rugs such as cefditoren pivoxil (meiacet), a new-third generation cephalosporin antibiotic which shows important activity over a large part of the pathogens causing skin and respiratory tract infections, including Gram-positive and Gram-negative bacteria has been suggested to cause significantly hypo-carnitinemia. Also, many studies have shown that carnitine is critical in fetal growth and fetal maturation during pregnancy. So, this study was performed to evaluate the genetic effect of cefditoren pivoxil alone or plus carnitine on embryonic toxicity, micronuclei formations and chromosomal aberrations in the pregnant females and their embryos throughout pregnancy. Pregnant female mice were administrated orally with three dosing regiments of meiacet (5, 10 and 20 mg/kg) and meiacet plus carnitine (5, 10 and 20mg/kg) twice daily for 14 days from day 3 to day 17 of pregnancy and on day 18 of pregnancy. Pregnant females were killed and examined for evidence of embryonic toxicity and cytogenetic effects including micronuclei formation and chromosomal aberrations in the maternal and embryonic cells.

It was observed that there were significant increases in the frequencies of embryonic toxicity, the micronucleus formation and the chromosomal aberrations in pregnant female mice as well as their embryos treated with the three doses of meiacet (5,10 and 20mg/kg) in dose dependent-manner but this increase in the low dose (5mg/kg) was close to the control group. On the other hand the results showed that in the three doses of meiacet plus carnitine (5, 10 and 20+5mg/kg) the frequencies of embryonic toxicity, micronucleus formation and chromosomal aberrations were also increased significantly over the control group but these increase were decreased significantly comparing with the groups of meiacet alone and became relatively similar to those in controls in the low dose of meiacet plus carnitine (5+5 mg/kg).

The results suggest that meiacet seems to be safe at the low dose but by increasing its concentration it caused genetic and embryonic toxicity, on the other hand, the administration of pregnant females with meiacet plus carnitine caused significant decreases in the frequencies of embryonic toxicity, micronuclei formation and chromosomal aberrations when comparing with the pregnant females administrated with meiacet alone and the frequency of the low dose of meiacet plus carnitine become in the limit of the control. This is may be due to that the administration of meiacet especially in high doses during pregnancy caused a decrease in the concentration of carnitine under the normal level and this decrease is dose-dependent The administration of carnitine is responsible for removing toxic substances from the body and improves embryonic and fetal growth.

Key words: Meiacet, cefditoren pivoxil, micronucleus test, embryonic toxicity, chromosomal aberrations, mice, embryos.

Introduction:

Antibiotics are chemical substances that in dilute solution can inhibit the growth of microorganisms or destroy them with little or no harm to the infected host. Early antibiotics were natural microbial products, but chemists have modified the structures of many to produce semisynthetic and even wholly synthetic ones. Antibiotics produced by certain bacteria, fungi and other organisms. Antibiotics may be broad-spectrum (active against a wide range of pathogens) or specific active against one, or one class).

Meiact (cefditoren pivoxil) is a new-third generation cephalosporin antibiotic that has recently been approved, shows important activity over a large part of the pathogens causing skin, soft tissue and respiratory tract infections, including Gram-positive and Gram-negative bacteria. Cefditoren pivoxil has also been shown to be stable against hydrolysis by many common beta-lactamases including penicillinases and some cephalosporinases.

Data from in vitro studies and clinical trials show this antibiotic as an oral formulation with an intrinsic activity against *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* equivalent to that of other third-generation cephalosporins administered via parenteral, like cefotaxime or ceftriaxone, thereby placing its maximal benefits mainly in the treatment of ambulatory infections¹.

The bactericidal activity of cefditoren results from the inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPS).

Cefditoren pivoxil (meiact) is hydrolyzed to its active component, cefditoren, and causes the subsequent formation of pivalate. Following multiple doses of cefditoren pivoxil 70% of the pivalate is absorbed and eliminated as pivaloyl carnitine leading to carnitine deficiency².

Carnitine is a derivative of the amino acid and is found in nearly all cells of the body. Its name is derived from the Latin *carnus* or *flesh*, as the compound was isolated from meat^{3,4}.

Carnitine plays a critical role in energy production. It transports long chain fatty acids into the mitochondria so they can be oxidized (burned) to produce energy. It also transports the toxic compounds generated out of this cellular organelle to prevent their accumulation. For these functions, carnitine is concentrated in tissues like skeletal and cardiac muscle that utilize fatty acids as a dietary fuel^{3,4}.

In general, carnitine occurs in two forms, known as D and L, there are mirror images (isomers) of each other. Only L-carnitine is active in the body and is the form found in food.¹

Healthy children and adults do not need to consume carnitine from food or supplement, as the liver and kidneys produce sufficient amounts from the amino acids lysine and methionine to meet dietary needs^{3,5}.

For genetic or medical reasons some individuals (such as preterm infants), cannot make enough carnitine^{3,6}.

Two types of carnitine deficiency states exist, primary carnitine deficiency is a genetic disorder of the cellular carnitine transport system and secondary carnitine deficiencies may occur due to certain disorders (such as chronic renal failure) or under particular conditions (e.g. use of certain antibiotics) that reduce carnitine absorption or increase its excretion⁷.

Carnitine interacts with pivalate conjugated antibiotics such as pivampicillin that are used in the long term prevention of urinary tract infections and also interacts with cefditoren pivoxil (meiact) which is used in the treatment of acute exacerbation of chronic bronchitis and skin structure infections⁸.

Chronic administration of these antibiotics increases the excretion of carnitine, which can lead to carnitine deficiency. Also, in the pregnant females during pregnancy the carnitine levels may be decreased under the normal level, so in these cases carnitine is approved to treat these conditions and this may have had a harmful effect to the pregnant females and their embryos.

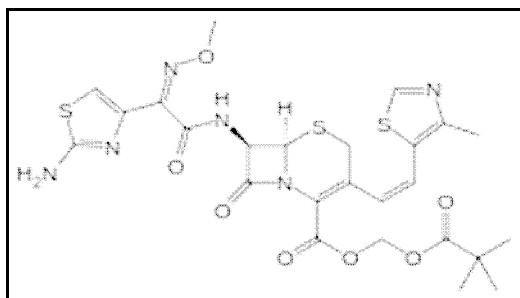
In fact there is no adequate and well control studies available that illustrates the safety use of cefditoren pivoxil (meiact) alone or in combination with carnitine if given orally throughout pregnancy. So, in

this study, we determined the effect of meiacet alone and meiacet plus carnitine on embryonic toxicity, micronucleus formations and chromosomal aberrations in pregnant female mice and their embryos.

Material and Methods:

Test substances

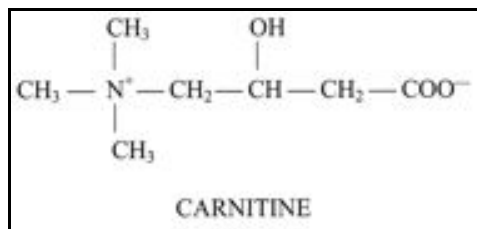
a) Mieact (cefditoren pivoxil) was provided by (Meiji Seika. Ltd Japan). Cefditoren pivoxil is available as off whit a tablet for oral administration and it is soluble in ethyl alcohol. The chemical name is (6R, 7R) 2,2-dimethyl propionyloxymethyl 7[(z)-2-(2-aminothiazol-4-YI)-2-methoxy-iminoacetamido]-3-[(2)-2-(4-methyl (thiozol-5-yl) ethenyl)] -8-0x0-5-thia-1-azabicydo[4.2.0]Oct-2-ene-2-carboxylate. Its chemical formula is $C_{25}H_{28}N_6O_7S_3$ and the molecular weight is 620.73. The structural formula of meiacet is.



b) Levocarnitine (L-carnitine):

Carnitine is a naturally available, quaternary ammonium compound, it is produced from amino acid, methionine and lysine.

Its chemical name is 3-carboxy-2-hydroxy-N, N, N-trimethyl-L-homocarnitine inner salt. Levocarnitine is a white crystalline; it is readily soluble in water. Its chemical formula is $C_7H_{15}NO_3$ and the molecular weight is 161.20. The chemical structural is:



Animals and treatments:

Dilutions of different concentrations were prepared by dissolving meiacet (cefditoren pivoxil) tablets in ethyl alcohol and dissolving carnitine tablets in distilled water.

Mature male and female Swiss mice weighing 26-30g were used; female mice were mated with males (3:1) overnight and examined in the next morning for a vaginal plug. The day on which a vaginal plug was found was considered the day 1 of pregnancy.

The pregnant females were divided into seven groups (10/group) as following:

The first group of pregnant females were administrated orally with a single dose of meiacet (5mg/kg) twice daily. The second group of pregnant females were administrated orally with a single dose of meiacet (10mg/kg) twice daily.

The third group of pregnant females were administrated orally with a single dose of (20mg/kg) twice daily. The fourth group of pregnant females were administrated orally with a single dose of (5+5mg/kg) meiacet plus a single dose of carnitine twice daily.

The fifth group of pregnant females were administrated orally with a single dose of meiac plus a single dose of carnitine (10+5mg/kg) twice daily.

The six group of pregnant females were administrated orally with as single dose meiac plus a single dose of carnitine (20+5mg/kg) twice daily.

The seven group of pregnant females were administrated orally with the same volume of distilled water served as a control group.

All the pregnant females were administrated orally from the day 3 to day 17 of pregnancy and on the day (18) of pregnancy the pregnant females in all groups were sacrificed by cervical dislocation for studying embryonic toxicity and cytogenetic effects (micronuclei formation and chromosomal aberrations) in maternal and embryonic cells.

Methods:

I. Embryonic toxicity:

On day 18 of pregnancy, the females were sacrificed by decapitation, the uterus was opened and the total number of embryos, number of live, dead and resorption embryos were recorded.

II. Micronucleus assay:

a) In females:

The females were sacrificed by cervical decapitation on day (18) of pregnancy. For each treatment five females were used in different groups. Bone marrow smears and staining were done following the method of [9]. Briefly, both the femora were removed and the bone marrow was flushed out into a centrifuge tube with 1% sodium citrate solution. The bone marrow cells were dispersed by gentle pipetting and centrifuged. The cell pellet was re-suspended in a small volume of 5% fetal calf serum. A drop of this suspension was smeared on a clean slide, air-dried fixed in absolute methanol for 15 min and stained with 5% Giemsa.

500 erythrocytes were examined for the presence of micronuclei (MN).

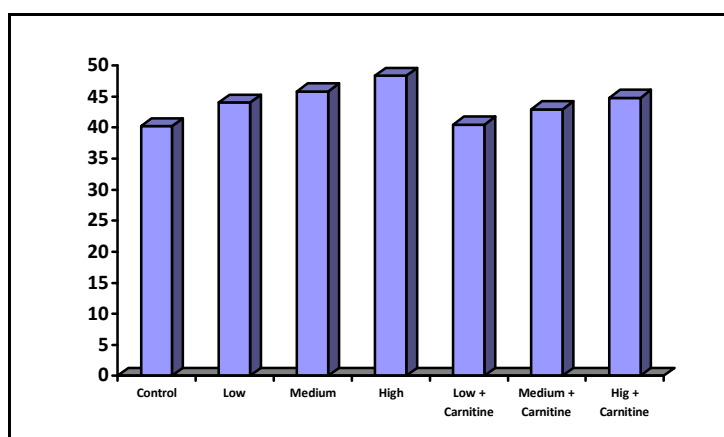


Fig (1): Micronudeated polychromatic erythrocytes (MnPCEs) of pregnant female mice exposed to several concentration of meiac and meiac plus carnitine (mean \pm S.D.)

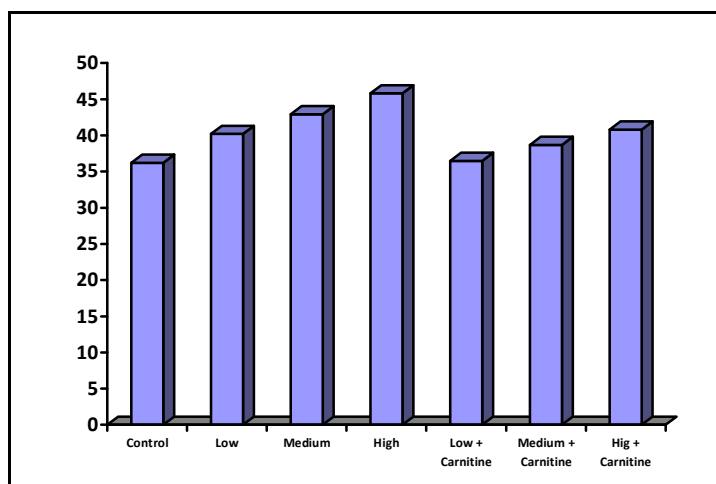


Fig (2): Micronucleated polychromatic erythrocytes (MnPCEs) of embryos resulted from mothers exposed to several concentration of meiact and meiact plus carnitine (mean \pm S.D.)

b) In embryos:

Embryos were taken on day (18) of pregnancy. Blood smears were taken from each embryo according to the method of¹⁰. Briefly, blood smears were taken from the tail of the embryo and the blood was re-suspended in a small volume of 5% fetal calf serum. A drop of suspension was smeared on a clean slide air dried fixed in absolute methanol and stained with 5% Giemsa stain. 500 cells were examined for the presence of micronuclei (MN).

III. Chromosomal aberrations assay:

a) In bone marrow cells (pregnant females)

Chromosomes from bone marrow cells were prepared according to the method of¹¹. Bone marrows were collected in T.C.M. 99 culture media and colchicine was added to the tube (2mL of 0.05 colchicine). Then, the cells were incubated at 37°C for 90 minutes. After centrifugation, 5mL of hypotonic solution was added and the pellet suspended and incubated at 37°C for 30 minutes. After centrifugation the cells were fixed in freshly prepared 3:1 methanol-glacial acetic acid then, two or three drops of cell suspension were dropped on a clean slide covered with cold ethanol and the slides were stained with 10% Giemsa stain.

b) In embryonic cells (embryos):

On day 18 of pregnancy, embryos were prepared cytogenetically according to the method of¹² with minor modifications. Embryonic livers were incubated in T.C.M. media containing 0.1 mg/ml colcemid for 90 min at 37°C and centrifuged, after centrifugation 5mL of hypotonic solution of 0.56% KCl were added to the pellet. The cells were re-suspended in the hypotonic solution for 15 min at 37°C, 5mL freshly prepared fixative (3 methanol:1 glacial acetic acid) were added. Two or three drops of cell suspension were dropped to the surface of cold clean slide, after dryness, the slides were stained with 5% Giemsa stain.

50 metaphase spreads were examined for each female and embryo. Structural and numerical aberrations were recorded.

Statistical analysis:

The incidences of resorption, dead and live embryos between experimental and control animals were calculated non-parametrically using wilcoxon's rank sum test¹³.

The data of chromosomal aberrations and micronucleus test in the pregnant females and embryos were subjected to analysis of variance (ANOVA) according to¹⁴. Least significant differences were used to compare between means according to¹⁵ at probability 5%.

Results:**1- Maternal Observations:**

Pregnant females administrated with different doses of meiact (5,10 and 20mg/kg) throughout pregnancy from day 3 to day 17 of pregnancy showed no signs of illness or abnormal behaviour and appeared more or less normal on gross observation.

However pregnant females administrated with different doses of meiact plus carnitine throughout pregnancy from day 3 to day 17 of pregnancy showed no signs of illness or abnormal behavior but appeared healthier on gross observation if compared with the groups treatment with meiact alone.

Moreover, there were no treatment-related effects on the fertility index in both groups of meiact and meiact plus carnitine comparing with the controls.

2- Embryonic toxicity:

Treatments with meiact (5, 10 and 20 mg/kg) during pregnancy from day (3) to day (17) of pregnancy induced a dose-related increase in the number of resorptions and in the number of dead embryos compared with the control but these increases were close to the limit of the control in the group of females treated with the low dose of meiact. Also, the treatments with meiact with the three doses caused dose related reduction in the numbers of live embryos but this reduction in the low dose of meiact within the limit of control.

On the other hand, in the groups of pregnant females treated with the three different doses of meiact plus carnitine (5,10 and 20+5 mg/kg) there was a little increased in the number of resorptions and dead embryos and decreased in the number of live embryos compared with the control but these effects decreased as compared with the groups treated with meiact alone and became close to the values of control group especially in the group of low dose of meiact plus carnitine (5mg/kg) the values of resorptions, dead and live embryos within the scope of control group table (1).

Table (1): Reproductive performance of pregnant female mice administrated orally with meiact alone or with carnitine from day 3 to day 17 of pregnancy.

Treatments	Dose (mg/kg)						
	Control	Low	Medium	High	Low + carnitine	Medium + carnitine	High + carnitine
No of females mated	10	10	10	10	10	10	10
No of pregnant females	10	10	10	10	10	10	10
Total no of embryos	87	83	80	77	86	84	81
No of resorption	1	1	2	3	0	0	1
%	1.1%	1.2%	2.5%	3.8%	0.0%	1.2%	1.5%
No of dead embryos	3	3	4	5	2	3	3
%	3.5%	3.6%	5%	6.5%	2.3%	3.6%	3.7%
No of live embryos	83	79	74	69	84	80	77
%	95.4%	95.2%	92.5%	89.6%	97.7%	95.2%	95%
Fertility index	100	100	100	100	100	100	100

Number given are the absolute number of embryos in that group with the indicated abnormality.

Micronucleus (MN) assay:**a) In pregnant females:**

The results of the percentage values of micronuclei and the distribution of PCE in the treated pregnant females and controls are given in table (2).

Table (2): The frequency of micro nucleated cells in pregnant females exposed to different concentration of meiact alone and meiact plus carnitine throughout pregnancy.

Treatments	Total no of MN	No of cells			Percentage of micronuclei per 500 cells
		1Mn	2Mn	3Mn	
Control	201 ^a ± 1.000	100	90	11	40.2 ^a ±0.2000
Low	220 ^c ± 1.000	105	100	15	44 ^c ±0.2022
Medium	229 ^e ± 1.000	107	105	17	45.8 ^e ±0.2000
High	240 ^f ± 1.000	110	108	22	48.33 ^f ±0.6110
Low + carnitine	202 ^a ± 0.577	100	90	12	40.46 ^a ±0.110547
Medium + carnitine	215 ^b ± 0.577	105	95	15	42.933 ^c ±0.1155
High + carnitine	224 ^d ± 1.000	110	98	16	44.800 ^d ±0.2000

Mean ± S.D. at P < 0.05

The pregnant females treated with three doses of meiact (5, 10 and 20mg/kg) from day 3 to day 17 of pregnancy showed significant increased in the percentage of micro nucleated cells and these increases were dose dependent and these increases were lower in the group of females treated with the usual low dose of meiact (5mg/kg) if compared with the other treated groups and with the control.

On the other hand, in the group of females treated with the three doses of meiact plus carnitine (5, 10 and 20+5 mg/kg) the frequencies of micronuclei were decreased significantly when compared with the groups of females treated with meiact alone and the frequency of micronuclei in the group of the low dose of meiact plus carnitine (5+5mg/kg) was became in the limit of control group.

b) In the embryos:

The results of the percentage values of micronuclei and the distribution of PCE in the embryos resulted from treated females and controls are given in table (3).

Table (3): The frequency of micro nucleated cells in embryos resulted from treated females exposed to different concentration of meiact alone and meiact plus carnitine throughout pregnancy.

Treatments	Total no of MN	No of cells with			Percentage of micronuclei cells
		1Mn	2Mn	3Mn	
Control	181 ^a ± 1.000	100	81	-	36.2 ^a ±0.2000
Low	210 ^c ± 1.000	111	90	-	40.2 ^c ±0.2000
Medium	214.67 ^e ± 0.577	120	94	-	42.93 ^e ±0.11547
High	229 ^f ± 1.000	129	100	-	45.8 ^f ±0.2000
Low + Carnitine	182 ^a ± 1.000	102	80	-	36.46 ^a ±0.30551
Medium + carnitine	194 ^b ± 1.000	106	88	-	38.69 ^d ±0.4000
High + carnitine	204 ^d ± 1.000	113	91	-	40.80 ^d ±0.2000

Mean ± S.D. at P < 0.05.

Generally, in the embryos obtained from treated females with meiact alone the frequencies of micro nucleated cells were increased significantly in the three doses when compared with the control group and these increases were dose-dependent but these increases were lower in the group of embryos resulted from females treated with the low dose of meiact (5mg/kg) compared with the other treated groups of females.

On the other hand, the frequencies of micro nucleated cells in the embryos resulted from pregnant females treated with the (low, medium and high) doses of meiact plus carnitine decreased significantly from the embryos resulted from the treatments with meiact alone and these decreases were in the same limit of the control group in the low dose of meiact plus carnitine (5+5mg/kg).

The distributions of micronuclei were different between pregnant females and embryos. In the pregnant females the majority of cells containing one, two and three micronuclei but in the embryos the majority of cells were containing only one and two micronuclei Table (2 and 3).

Chromosomal aberrations:

a) In the pregnant females:

Table (4) present the results of chromosomal aberrations analysis in the treated pregnant females with the three doses of meiac alone or in combination with carnitine (5, 10 and 20 mg/kg) from day 3 to day 17 of pregnancy. In all treated groups of females there were significant increases in the total number of structural aberrations comparing with the control group and this increase was lower in the low dose of meiac alone comparing with the other treated groups.

Table (4): The effect of oral administration of meiac alone and meiac plus carnitine on pregnant females

Groups	Structural aberrations							Numerical aberrations			
	Gaps	Breaks	Deletion	Centromeric attenuation	Endometosis	Fragments	T.S.A	Hypo <40	Hyper >40	Poly-ploidy	T.N.A.
Control	5.00 ^a ± 0.577	2.67 ^a ± 0.333	3.67 ^a ± 0.333	4.67 ^a ± 0.333	5.33 ^a ± 0.333	0.67 ^a ± 0.333	22.00 ^a ± 0.577	4.00 ^{ab} ± 0.577	3.00 ^a ± 0.00	0.33 ^a ± 0.333	7.00 ^a ± 0.577
Low	5.33 ^a ±.333	3.00 ^a ± 0.000	4.00 ^a ± 0.577	5.00 ^{ab} ± 0.577	6.00 ^{ab} ± 0.000	7.67 ^{bc} ± 0.333	25.00 ^b ± 0.000	3.33 ^a ± 0.667	3.67 ^{ab} ± 0.33	0.679 ^a 0.333	7.67 ^a ± 0.333
Medium	5.67 ^{ab} ± 0.333	3.33 ^a ± 0.333	4.33 ^a ± 0.333	5.67 ^b ± 0.333	6.67 ^{bc} ± 0.383	1.333 ^{ab} ± 0.333	27.000 ^c ± 0.577	4.67 ^{ab} ± 0.333	5.00 ^b ± 0.577	1.33 ^a ± 0.333	11 ^c ± 0.577
High	6.67 ^b ± 0.333	5.33 ^b ± 0.333	4.67 ^b ± 0.333	6.00 ^c ± 0.000	7.00 ^c ± 0.000	2.67 ^d ± 0.333	32.33 ^d ± 0.333	5.33 ^b ± 0.333	5.67 ^c ± 0.333	2.67 ^b ± 0.333	13.67 ^d ± 0.333
Low + carnitine	5.00 ^a ± 0.000	2.67 ^a ± 0.333	4.00 ^a ± 0.00	4.33 ^a ± 0.333	5.33 ^a ± 0.333	1.00 ^{ab} ± 0.667	22.33 ^a ± 0.667	4.00 ^{ab} ± 0.577	3.00 ^a ± 0.577	0.00 ^a ± 0.00	7.00 ^a ± 0.00
Medium + carnitine	5.33 ^a ± 0.333	3.33 ^a ± 0.323	4.00 ^a ± 0.00	4.33 ^a ± 0.333	5.67 ^a ± 0.333	1.67 ^b ± 0.333	24.67 ^b ± 0.667	4.33 ^{ab} ± 0.333	4.00 ^{ab} ± 0.000	1.33 ^{ab} ± 0.667	9.67 ^b ± 0.333
High+carnitine	5.67 ^{ab} ± 0.333	4.67 ^b ± 0.333	4.33 ^a ± 0.333	5.33 ^a ± 0.333	6.67 ^{bc} ± 0.333	2.000 ^{cd} ± 0.000	28.67 ^c ± 0.886	5.00 ^{ab} ± 0.577	5.00 ^{bc} ± 0.577	1.33 ^{ab} ± 0.667	11.500 ^c ± 0.5000

Means ± S.E. of different letters (a,b,c,d,e,f) in the same column are significantly different P < 0.05.50 metaphase cells were examined from each animal.

Moreover, in the groups of females treated with the low dose of meiac the frequency of the total numerical aberrations in the limit of control group. While in the medium and high groups of meiac the frequencies of numerical aberrations increased significantly comparing with the control group.

On the other hand, in the groups of pregnant females treated with the three doses of meiac plus carnitine the frequencies of chromosomal aberrations (structural and numerical) were decreased significantly if compared with the treated groups of meiac alone and in the group of females treated with the low dose of meiac plus carnitine the frequencies decreased and became in the limit of control group.

b) In the embryos:

Table (5) present the results of chromosomal aberrations analysis in embryonic cells (18 dg) resulted from treated females with meiac alone and meiac plus carnitine from day (3) to day (17) of pregnancy (5,10 and 20+5 mg/kg). The results showed that the frequencies of structural chromosomal aberrations in the three doses of meiac increased significantly and these increases were less in the groups of embryos resulted from females treated with the low dose of meiac when compared with the control group.

Table (5): The effect of oral administration of meiact alone and meiact plus carnitine on embryos resulted from treated pregnant females.

Groups	Structural aberrations							Numerical aberrations			
	Gaps	Breaks	Deletion	Centromeric attenuation	Endometosis	Fragments	T.S.A	Hypo <40	Hyper >40	Poly-ploidy	T.N.A.
Control	3.67 ^a ± 0.323	2.00 ^a ± 0.000	3.33 ^a ± 0.333	4.00 ^a ± 0.572	5.00 ^b ± 0.000	1.33 ^a ± 0.333	19.33 ^a ± 0.667	3.33 ^a ± 0.333	2.67 ^a ± 0.333	0.00 ^a ± 0.000	6.00 ^a ± 0.57
Low	4.67 ^{ab} ± 0.333	2.67 ^{ab} ± 0.333	3.67 ^{ab} ± 0.333	4.33 ^a ± 0.333	5.00 ^b ± 0.000	2.00 ^{ab} ± 0.000	22.67 ^b ± 0.667	4.00 ^{ab} ± 0.000	3.00 ^{ab} ± 0.000	0.33 ^a ± 0.333	7.33 ^a ± 0.333
Medium	5.00 ^{bc} ± 0.577	3.33 ^{bc} ± 0.333	4.00 ^{ab} ± 0.577	4.67 ^a ± 0.333	4.33 ^{ab} ± 0.333	3.33 ^c ± 0.667	24.67 ^c ± 0.667	4.00 ^{ab} ± 0.577	3.67 ^{bc} ± 0.333	1.67 ^b ± 0.333	9.33 ^{cd} ± 0.332
High	6.00 ^c ± 0.00	4.67 ^a ± 0.333	5.33 ^c ± 0.333	5.33 ^a ± 0.667	6.00 ^c ± 0.000	3.33 ^c ± 0.333	30.67 ^d ± 0.333	5.33 ^c ± 0.333	5.00 ^d ± 0.00	2.00 ^b ± 0.000	12.33 [±] 0.333
Low + carnitine	3.67 ^a ± 0.333	3.00 ^{ab} ± 0.577	3.67 ^{ab} ± 0.333	4.00 ^a ± 0.00	4.00 ^a ± 0.577	1.33 ^a ± 0.333	19.67 ^a ± 0.333	3.67 ^a ± 0.333	2.67 ^a ± 0.333	0.33 ^a ± 0.333	6.67 ^a ± 0.333
Medium + carnitine	4.67 ^{ab} ± 0.333	3.33 ^b ± 0.333	3.67 ^{ab} ± 0.667	4.33 ^a ± 0.333	3.67 ^a ± 0.333	2.67 ^{bc} ± 0.333	22.33 ^b ± 0.333	4.00 ^{ab} ± 0.577	3.33 ^{ab} ± 0.333	1.00 ^{ab} ± 0.577	8.33 ^{bc} ± 0.667
High + carnitine	5.33 ^{bc} ± 0.333	4.33 ^{cd} ± 0.333	5.00 ^{bc} ± 0.000	04.67 ^a ± 0.333	5.00 ^b ± 0.00	2.67 ^{bc} ± 0.333	26.67 ^c ± 0.882	5.00 ^{bc} ± 0.000	4.33 ^{cd} ± 0.333	1.00 ^{ab} ± 0.577	10.00 ^d ± 0.000

Means ± S.E. of different letters (a,b,c,d,e,f) in the same column are significantly different $P < 0.05$. 50 metaphase cells were examined from each animal.

While in the group of embryos resulted from treated females with the low dose of meiact there were no significant differences in the frequencies of numerical aberrations compared with the control. On the other hand, the frequencies of numerical aberrations in the two doses of meiact (medium and high) increased significantly compared with the control group.

Moreover, in the groups of embryos resulted from pregnant females treated with three doses of meiact plus carnitine the frequencies of chromosomal aberrations (structural and numerical) were decreased significantly when comparing with the treated groups of meiact alone and these decreases were in the limit of the control group in the group of embryos resulted from low dose of meiact plus carnitine (5+5mg/kg).

Discussion:

Pivalic acid (trimethylacetic acid, $C_5H_{10}O_2$) has been commonly used to create prod rugs to enhance drug delivery. Pivalate-generating prod rugs have been suggested to cause clinically significant hypocarnitinemia^{17,18}.

Carnitine interacts with pivalate-conjugated antibiotics such as a new third generation oral cephalosporin cefditoren pivoxil (meiact) that are used in the long-term prevention of acute exacerbations of chronic bronchitis, mild-to-moderate acute maxillary sinusitis (respiratory tract infections) and skin structure infections. The administration and the prolonged use of this antibiotic increase the excretion of pivaloyl-carnitine, which can lead to carnitine depletion¹⁹.

Carnitine plays a critical role in energy production and also carnitine transport the toxic compounds that produced in the body and prevent their accumulation. So the lower level of carnitine increased the accumulation of these toxic compounds inside the body leading to harmful effects (I-2).

Moreover, during pregnancy the plasma carnitine concentrations were also decreased under the normal level.

This may be as a result that is the fetus incapable for synthesis of carnitine thus, carnitine concentrations in fetal tissues are greatly dependent on maternal carnitine status and the rate of placental transfer²⁰⁻²¹.

Previous studies demonstrated the placenta fetal interrelationship in carnitine metabolism during pregnancy and suggested that supplementation of carnitine. During pregnancy may be beneficial to both mother and embryos²²⁻²³.

In fact there is no adequate and well control studies suggested the Genetic toxicity of cefditoren pivoxil (meiact) a new third-generation oral cephalosporin antibiotic on pregnant females and embryos if used alone or in combination with carnitine throughout pregnancy.

So, in the present study we evaluate the cytogenetic and embryo toxic effects of meiact alone or plus carnitine if used in three dosing regimes (low, medium and high) for 14 days throughout pregnancy.

In the present study, we found that the treatments of the pregnant females from the day (3) to day (17) of pregnancy with the low dose of meiact (5mg/kg) caused a slight increase in the number of dead and resorbed embryos and decrease in the number of live embryos but these effects close to the limit of control group. Also in the other treated groups with cefditoren pivoxil (10 and 20mg/kg) there were increased in the number of dead and resorbed embryos and decreases in the number of living embryos and these effects were more frequent in these two groups compared with the low dose group.

The fertility indexes in all groups were not affected by the treatments and were the same as the controls.

However, in the groups of pregnant females treated with meiact plus carnitine (cefditoren pivoxil) there were slight decreases in the number of dead and resorbed embryos and increases in the number of living embryos in the three treated groups (5, 10 and 20+5mg/kg) as compared with the three treated groups with meiact alone and these effects were in the scope of control group especially in the group treated with the low dose of cefditoren pivoxil plus carnitine.

Our results were agreement with²⁴ who observed that after oral administration of cefditoren pivoxil at oral dose 90 mg/kg/day to rabbits resulted severe maternal toxicity that resulted in fetal toxicity and abortions.

Also, positive results were obtained by²⁴ who found that after oral administrations in rats with cefditoren pivoxil at oral doses up to 1000mg/kg/day the fertility index was not affected.

However negative results were obtained by²⁴ who observed that the oral administration in rats with a dose of 1000mg/kg/day this tested dose did not cause teratogenic effects.

Also negative results were observed by²⁴ who found that in a postnatal development study in rats, cefditoren pivoxil produced no adverse effects on postnatal survival physical and behavioral development at doses up to 750 mg/kg/day.

Moreover positive results were obtained by²⁴ who found that carnitine did not induce teratogenic effects on the pregnant rats if given orally at a dose 400 mg/kg/day.

Also, positive results were obtained by²⁵ who found that supplementation with L-carnitine during pregnancy may be beneficial to both mother and fetus. Carnitine may increase fatty acid and glucose oxidation and improve fetal energy metabolism, which are critical to embryonic and fetal development.

Moreover, positive results were given by²⁶ who observed that the supplementation with carnitine during pregnancy may increase birth weight and postnatal growth also carnitine work as an antioxidant and prevent the embryonic toxicity.

In the present study the long-term oral administrations of cefditoren pivoxil throughout pregnancy with the low dose (5mg/kg) from day (3) to day (17) of pregnancy caused a slight significant increase in the chromosomal aberrations in the bone marrow maternal cells and embryonic cells and caused also a slight significant increase in the percentage of micronuclei in both maternal and embryonic cells.

However, the oral administrations of cefditoren pivoxil with the medium and high doses (10 and 20mg/kg) caused more frequent significant increases in the chromosomal aberrations and micronuclei formations in the bone marrow and embryonic cells.

These findings were agreement with²⁴ who observed that in Chinese hamster lung cells, chromosomal aberrations were produced by cefditoren pivoxil. Subsequent studies showed that the chromosomal aberrations were due to the release of formaldehyde from the pivoxil ester moiety in the in vitro assay system.

Similar results were obtained by²⁷ who found that the treatments with antibiotics containing pivalic acid may be produced secondary-carnitine deficiencies which caused a harmful effect especially to the pregnant females.

Also, positive results were obtained by²⁸ who found that in animal models, administration of pivalate drugs is associated with pivaloyl carnitine accumulation and this accumulation may be toxic.

However, negative results were obtained by²⁴ who found that neither cefditoren nor cefditoren pivoxil produced chromosomal aberrations when tested in vitro human peripheral blood lymphocyte assay, or in the in vivo mouse micronucleus assay.

Also, in the current study, the oral administration of cefditoren pivoxil (meiact) in combination with carnitine in a dose of (5+5mg/kg) caused a significant decrease in the frequencies of chromosomal aberrations and micronuclei in the maternal cells and embryonic cells compared with low dose of meiact alone and became in the range of the control group.

Moreover, the oral administrations of cefditoren pivoxil plus carnitine with medium and high doses (10 and 20+5mg/kg) caused significant decreases in the frequencies of chromosomal aberrations and micronuclei formation in the maternal and embryonic cells compared with the groups of meiact alone and also became close to the control group.

These results are agreement with²⁹ who found that several drugs such as pivalic acid containing antibiotics are associated with decreased in carnitine levels and this conditions may be caused a harmful effects so carnitine has been proposed as a treatment for many conditions because it acts as an antioxidant and he also found that carnitine fight harmful particles in the body known as free radicals which damage cells and tamper with DNA. Antioxidants can neutralize free radicals and may reduce some of the damage they cause.

Also, positive results were obtained by³⁰ who found that during pregnancy the mean whole blood and plasma carnitine levels are already lower than those of controls and he found that the carnitine supplementation in pregnancy in sufficient doses avoids the increase of plasma FFA, S, which are cause of insulin resistance and consequently the gestational diabetes which caused toxic effects to the mothers and embryos.

Moreover, positive results were obtained by³¹ who observed that the short term administration of cefditoren pivoxil results decreased in the levels of plasma carnitine and increased net losses of total carnitine. The decreases of carnitine concentration was dependent on the dose of cefditoren and the subject gender (decrease from 44.8 ± 10.9 Mmol/L to 9.2 ± 1.9 Mmol/L) in male patients and from (32.5 ± 5.4 Mmol/L to 6.3 ± 1.7 Mmol/L) in female patients after 14 days of 400mg meiact twice daily.

Conclusion:

In conclusion, the current study indicated that meiact (cefditoren pivoxil) seems to be safe during pregnancy if taken in a low dose but by increasing its concentration it may be caused genetic toxicity this is may be due to the administration of pivalate prod rugs (meiact) has been associated with decreases in plasma carnitine concentration, (hypocarnitine) and increasing of formaldehyde formation (low, medium and high).

However, the administration of meiact with different doses plus carnitine caused healthy maternal and embryonic behavior and decreased in the mutagenic and cytotoxic effects of meiact and the frequencies of genetic toxicity became close to the control group especially in a low dose. This may be due to the oral administration of carnitine plus meiact increased carnitine concentrations in the pregnant females then the placenta capable of the transport of carnitine to the embryo in the early stages of pregnancy this transfer may be increased the fatty acid and glucose oxidation remove the toxic compounds produced from the prolonged administration of antibiotic and improve embryonic and fetal growth.

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