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Original Article

To determine morphology after administration of tetracycline and zinc chloride in adult albino rats and prenatal conceptus.

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Abstract:

Introduction: Administration of tetracycline therapy is controversial during growing age as stated by earlier workers. However the effect of simultaneous administration of tetracycline & $Zncl_2$ on pre-natal concepts has remained undocumented.

Objective: To compare the effect on conceptus after maternal ingestion of tetracycline and $Zncl_2$ by prospective experimental animal study model using adult albino rats.

Methodology: Tetracycline & $ZnCl_2$ was administrated to pregnant female albino rats two times therapeutic dose for 10 days. (from day 8 to day 18 of pregnancy.) Each animal was weighted on day 1, day 8 & day 18 of pregnancy. Abortion resulted on day 18^{th} of pregnancy. Each group of pregnant animal were scarified on day 18 of gestation by overdose of either anesthesia, abdomen opened, uterus & both cornu containing conceptus identified, removed, there weight recorded, crown rump length was measured & was compared with similar value of control animals. The results were statistically analyzed to find out the significance.

Result: Maternal administration of tetracycline decreased maternal bodyweight to 38.4 ±0.9gm. However simultaneous administration of $ZnCl_2$ maintained bodyweights to 41.4 ±0.7gm. The body weight and crown rump length (CR Length) in conceptus decreased by 4.52 ±0.10gm and 3.06 ±0.09 cm respectively. That tetracycline & $ZnCl_2$ administration maintained the body weight & CR length by 5.46 ±0.09 gm and 3.79 ±0.13gm respectively.

Conclusion: Pre-natal administration of Tetracycline alone cause reduction in maternal body weight as well as reduction in body weight & CR length of rat conceptus. However simultaneous $Zncl_2$ maintain body weight & CR length leading to growth of the rat conceptus.

Keywords: Tetracycline, $ZnCl_2$, Rat Conceptus. Crown Rump Length (CR Length).

Introduction:

Tetracycline was originally made from bacteria of the streptomyces type[1]. Tetracycline is one of drug having an extended spectrum of antimicrobial activity. It has best bioavailability 80-95% serum level and is remained widely in body fluids & tissues[2]. It has better long serum half-life to suit twice daily dosing[3]. Tetracycline was patented in 1953 and came in commercial use in 1978[4]. It is on WHO list of essential medicines, the most effective medicines needed in a health system[5]. The Tetracycline is one of the most commonly use antibiotics now a days for different kinds of infections. It is the first line therapy for Rocky Mountain Spotted fever (Rickettsia), Lyme disease, Q fever, Psittacosis, Mycoplasma Pneumoniae and Syphilis[6]. Concomitant with its wide range of activity and common usage, it inherits many toxic effects on liver & epiphysial growth plate in experimental animals[7.] Tetracycline inhibits protein synthesis by blocking the attach-

ment of charged aminoacyl-tRNA to the a site on the ribosome. Tetracycline binds to the 30S and 50S subunit of microbial ribosomes to prevents introduction of new amino acids to the nascent peptide chain.[8]

Zinc is one of the essential trace minerals and is necessary for synthesis of DNA and RNA proteins and functions as catalyst for several enzymes. Zinc stabilizes the structure of nucleic acid protein and thereby preserves the integrity of intracellular organelles such as mitochondrion. Zinc plays many significant roles in metabolism as component of many metalloenzymes and transcription factors.[9]

Objective:

The study is undertaken to determine the effect of Tetracycline & $Zncl_2$ on the crown rump length & body weight of juvenile laboratory albino rat offspring's to confirm the possibility of Arora's speculation & find out it's magnitude.

Methodology:

Thirty spontaneously ovulating female & 15 fertile male albino rats of 16-18 weeks age were taken from animal house of basic medical science institute, Jinnah Postgraduate Medical Centre Karachi. The female rats were mated with of same strain according to the method described by.[10] Thus one male rat was mated with two female rats in a separate cage. On next day one female rat were examined for signs of mating. Such a blood strained vagina or vaginal plug of mucoid greenish white material. Presence of both or any of these signs were considered a day zero of pregnancy (Fig-1). All pregnant rats were weighed on day one of pregnancy. The mean gestation period of albino rat is 21 to 23 days.[7] Thirty prenatal-natal concepts were randomly selected irrespective of the age to make three groups A,B & C. Each group comprised of 10 animals.

Group A:

Control pre-natal conceptus, their mothers were injected normal saline in equal volume,[11] i.e. 0.1ml intra-peritoneally twice daily for 18 days from day 1 to day 18 of pregnancy.

Group B:

Pre-natal conceptus, their mothers were injected Tetracycline developed in Bayer research laboratories AG German at a dose of 20mg/kg body weight,[12] i.e. 0.12 mg of drug dissolved into 0.1ml of the solvent, intra-peritonially twice daily for 18 days from day 1 to day 18 of pregnancy.

Group C:

Pre-natal conceptus, their mothers were injected Tetracycline at a dose of 20mg/kg body weight, i.e. 0.12mg of the drug dissolved into 0.1ml of the solvent and zinc chloride salt (developed in laboratory chemical in west Germany) at a dose of 120 µgm/kg bodyweight i.e. 7.4 µgm salt dissolved into 0.1ml of the solvent Intra-peritoneally 30 minutes before the administration of tetracycline twice daily for 18 days from day 1 to day 18 of pregnancy. Each animal weighted on day 1, day 8 & day 18 of pregnancy and their weight were recorded in proforma Table-I. The animals of each group were sacrificed on day -18 of gestation by an overdose of ether anesthesia, abdomen opened, uterus & both cornua containing offspring were identified and removed ,harvested, washed in normal saline. Their photographs were taken by special photographic camera, Fig-2. Each conceptus were weighed on Sartorius balance and their body weight were recorded in proforma, Table-2. specially designed for it. Their CR length were measured by measuring tape. Table-3. Morphometry was done and data was statistically analyzed by students "t" test to determine the statistical significance of results.[13]

Results: In present study, we observed the effects of tetracycline and $zncl_2$ on maternal and pre-natal conceptus body weight & CR length separately & simultaneously. The experimental body weight & crown rump length & their morphological changes were compared with control animals.

The mean body weight & CR length of experimental & control animals are given in Table-1,2,3 & Fig 1,2. Abortion resulted in mothers on day 18^{th} of gestation subsequently these mothers showed abrupt average weight reduction of 50 gm.

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THE BODY WEIGHT (G) DURING GESTATION BETWEEN CONTROL & EXPERIMENTAL ALBINO RATS.

The mean normal bodyweight in control animals at day -1, day-8 & day-18 was measured 40.5±1.52 gm. While tetracycline treated animals body weight was reduced to 38.4±0.9gm. Which is statistically highly significant (P<0.001). However simultaneous treatment of $zncl_2$ maintained the body weight loss to 41.4±0.7gm which is statistically nonsignificant (P<0.064).

THE BODY WEIGHT (G) OF ALBINO RAT CONCEPTUS BETWEEN PRE-NATAL CONTROL & TREATED ANIMALS.

The mean normal body weight in control conceptus was measured 5.44 ±0.12 gm, while in tetracycline treated conceptus reduced body weight to 4.52 ±0.10 gm which is statistically highly significant (P<0.001). However simultaneous $ZnCl_2$ treated animals maintained the weight to 5.46 ±0.09 gm, which is statistically nonsignificant (P>0.89).

THE CROWN RUMP LENGTH(CM) OF ALBINO RAT CONCEPTUS BETWEEN PRE-NATAL CONTROL & TREATED ANIMALS.

The mean normal CR length in control conceptus was measured 3.97 ±0.07 cm, While in tetracycline treated conceptus reduced CR length to 3.06 ±0.09 cm which is statistically highly significant (P<0.001). However simultaneous $ZnCl_2$ maintained the CR length to 3.79 ±0.13 cm, which is statistically nonsignificant (P>0.24).

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Discussion:

Tetracycline is a broad-spectrum antibiotic active against both gram-positive and gram-negative bacteria. It functions by inhibits protein synthesis by blocking the attachment of charged aminoacyl-tRNA to a site on the ribosome. Tetracycline binds to the 30S and 50S subunit of microbial ribosomes. Thus, it prevents introduction of new amino acids to the nascent peptide chain.[5] The action is usually inhibitory and reversible upon withdrawal of the drug[14].

Present study is therefore aimed to determine the effects of tetracycline and $ZnCl_2$ when administered separately and simultaneously during gestation of albino rat the effects on the mother and the conceptus during day 18 are assessed as the measurement of the body weight and CR length, as the parameter.

Our observation revealed that there is reduction in body weight and CR length in Group B tetracycline treated mothers was found to be 38.4 ±0.9 gm and in conceptus i.e. 4.52 ±0.10 gm & 3.06±0.09 cm respectively, as compared to Group A control mothers i.e. 40.5 ±1.52 gm and in conceptus i.e. 5.44±0.12 gm & 3.97 ±0.07 cm respectively. The results confirmed that reduction of the body weight and CR length were affected by the adverse effects of tetracycline. These findings are attributed mainly to reduction of body weight & CR length due to less intake of food. Our observations are in consistence with findings.[15] Since retarded conceptus growth can give rise humans to limb length discrepancy. Therefore tetracycline might cause disorder similarly[16], found that complete or partial growth retardation may result in limb length discrepancies.

The parameters shows statistically insignificant differences in body weight & CR length of the mother and the conceptus. The simultaneous administration $ZnCl_2$ in animals in Group-C the mother weighed 41.4 ±0.7 gm and the conceptus 5.46 ±0.09 gm and measured 3.79 ±0.13 cm respectively. When compared with age matched controls Group-A i.e. 40.5 ±1.52 gm, and 5.44 ±0.12 gm & 3.97 ±0.07 cm respectively. These findings confirmed the protective role of $ZnCl_2$. Our observations support those of WHO contributors[17]. Who found that zinc supplements help prevent disease and reduce mortality specially among children with low birth weight or stunted growth.

Abortion resulted in three mothers on 18th day of gestation subsequently these mothers showed abrupt average weight reduction of 50 gm. However the abortus was not available as eaten by the rat herself, Indicated by tell tale blood stains in the cage and grossly reduced dietary intake last day give the clue. And hysterotomy confirmed the abortion.

Conclusion:

Tetracycline retards the conceptus growth and mother body weight. ZnCl2 has the definite preventive role on the tetracycline retarding effect on the conceptus. ZnCl2 has confirmed exaggerated role in growth of length and weight in the conceptus.ZnCl2 effects on the conceptus are medicated by the increase in weight and intake of the mother.

The study shows that ZnCl2 effect on the enzyme system some how leads to the increased appetite of the mother. As indicated by significantly increased daily intake of the mother. The effect of the ZnCl2 on the appetite and satiety centers in the hypothalamus need to be further physiologically explored.

Three of cases on day 18th of gestation tetracycline caused late abortion in 30% animals.

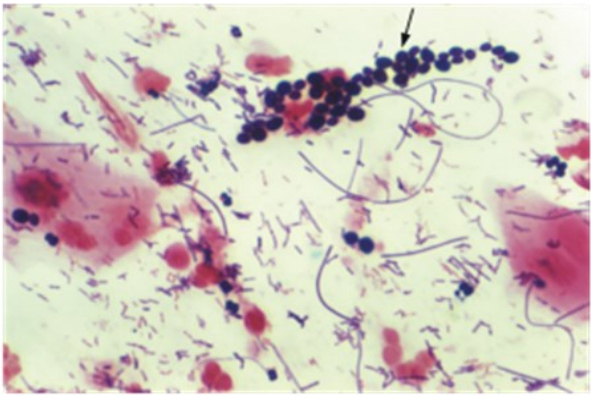

Fig-1. Photomicrograph of staining vaginal smear with H & E which contained spermatozoa to confirming pregnancy.

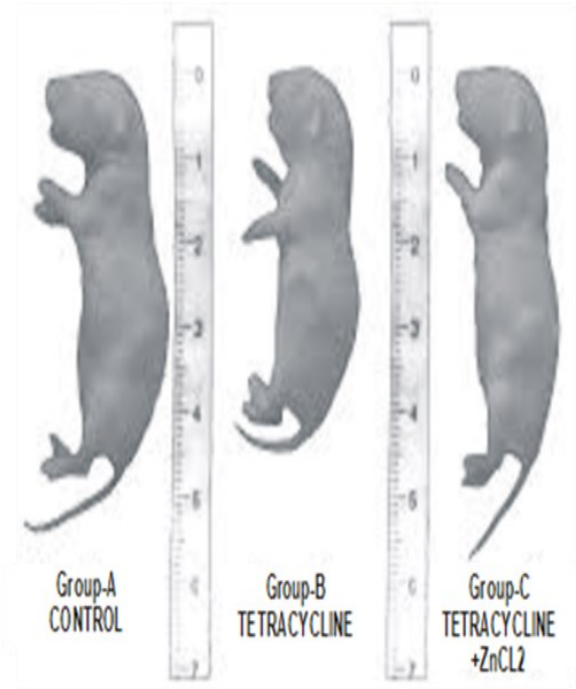

Fig 2. Photograph of Albino rat conceptus on 18 pre-natal day showing comparison of CR length between control group A, experimental groups B & C used in this study.

Table- 1: COMPARISON OF BODY WEIGHT (G) GAIN / LOSS DURING GESTATION BETWEEN CONTROL AND TREATED GROUPS

S.#	A (Control)				B (Tetracycline)				C (Tetracycline + $ZnCl_2$)			
	GD1 (I)	GD 8	GD 18 (F)	Wt. G/L	GD1 (I)	GD 8	GD 18 (F)	Wt. G/L	GD1 (I)	GD 8	GD 18 (F)	Wt. G/L
1	153	170	196	42	148	170	184	36	150	170	190	40
2	155	174	198	43	149	175	189	40	148	160	185	37
3	150	168	180	30	150	185	190	40	150	170	192	42
4	151	170	198	47	152	175	185	38	155	175	195	40
5	150	172	190	40	150	188	190	40	154	174	196	42
6	150	170	190	40	150	180	192	42	150	175	195	45
7	150	172	185	35	152	175	188	36	150	170	190	40
8	150	175	192	42	149	180	190	41	154	175	195	41
9	150	170	194	44	150	185	192	42	148	170	190	42
10	150	170	192	42	150	180	189	39	150	175	195	45
N	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Mean	150.9	171.1	191.5	40.5± 1.52	150	179.3	188.9	38.4± 0.9	150.9	171.4	192.3	41.4± 0.7
SD	1.728	2.131	5.681	4.813	1.247	5.618	2.643	2.96	2.54	4.671	3.529	2.41
SEM	0.54	0.67	1.79	1.52	0.394	1.776	0.835	0.936	0.795	1.477	1.11	0.76

Legend: Wt. G/L = Weight Gain / Loss, I= initial, F= Final, GD= gestational day, G= gram, SD= standard deviation, N=Total number of animals, SEM = Standard error of mean.

STATISTICAL ANALYSIS		
Group	**P Values**	**Significant / Non-Significant**
A vs B	< 0.035 ↓	Significant
A vs C	> 0.064 -	Non-Significant
B vs C	< 0.05 ↓	Significant

TABLE-2. COMPARISON OF BODY WEIGHT (G) OF ALBINO RAT CONCEPTUS BETWEEN PRENATAL CONTROL AND TREATED GROUPS

S.#	A (Control)	B (Tetracycline)	C (Tetracycline + $ZnCl_2$)
	GD 18	GD 18	GD 18
1	5.17	4.51	5.65
2	5.39	4.61	5.30
3	5.00	4.17	5.45
4	5.00	4.70	5.95
5	5.02	4.90	5.00
6	5.96	4.13	5.10
7	5.90	4.99	5.90
8	5.70	4.80	5.35
9	5.75	4.31	5.60
10	5.50	4.13	5.30
N	N=10	N=10	N=10
Mean	5.44 ±0.12	5.52 ±0.10	5.46 ±0.09
SD	0.378	0.325	0.315
SEM	0.119	0.103	0.0996

STATISTICAL ANALYSIS

Group	P Values	Significant / Non-Significant
A vs B	< 0.001 ↓	Highly Significant
A vs C	> 0.24 -	Non-Significant
B vs C	< 0.001 ↓	Highly Significant

Legend: Wt. G/L= Weight-Gain/Loss, I= initial, F= Final, GD= gestational day, G= gram, SD= standard deviation, N= Total number of animals, SEM = Standard error of mean.

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