Morphological and Skeletal Abnormalities Induced by α/β Arteether on Developing Chick Embryo

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Abstract

Introduction: Malaria continues to be one of the India's leading public health problem.α/β artether is one of the most common antimalarial drug used worldwide to treat chloroquine resistant malaria and malaria falciparum. The present study was designed to assess the teratogenic effects of α/β artether on developing chick embryo. **Material and Methods:** The study was performed on 300 fertilized eggs of white leg horn chicken.The eggs were divided in to five experimental groups A, B, C, D, E having 30 eggs each and five control groups a,b,c,d,e one each for every experimental group respectively having 30 eggs each. On 5th day of incubation eggs from experimental groups A, B, C, D and E were exposed to α/β artether with dose of 0.00039 mg, 0.000585 mg, 0.00078 mg, 0.00097 mg and 0.00117 mg whereas the control groups were treated with same amount of normal saline. **Results:** The results showed growth retardation and some significant morphological abnormalities like scanty feathers, subcutaneous hemorrhage and skeletal abnormalities like poor ossification of the bones, kyphosis and lordosis. **Discussion:** The drug is toxic specially when used in higher dose and for a long period. At present there is no alternative drug available for the treatment of chloroquine resistant malaria and malaria falciparum except α/β artether. Therefore α/β artether and other artemisinins should be used only after establishment of proper diagnosis in recommended dose only not in higher dose and not for a long duration.

Keywords: Artemisinins, kyphosis, lordosis, scoliosis, teratogenicity, α/β arteether

INTRODUCTION

The only method of attaining a comprehensive understanding of embryological processes is through the study and comparison of development in various animals, and the chick is one of the most satisfactory animals on which embryological laboratory work may be based.[1] The traditional strength of chicks embryos for studying development is that they are easily manipulated, and this character of the chicken has led to some major discoveries in embryological science.[2] Malaria a febrile illness spread through mosquito continues to be one of the world's leading public health problems including India. In India, malaria imposes an incredible socioeconomic burden on humankind and with six other diseases such as diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia are present 85% of global infectious disease burden.[3] In the southeast Asian locality of the WHO, out of 1.4 billion individuals living in 11 countries, approximately 1.2 billion individuals are presented to the danger of malaria and majority of whom live in India. India reports approximately two million cases and 1000 deaths attributable to malaria yearly.[4]

α/β arteether is the concentrate of artemisia plant found in China also called Qinghaosu. It has the most rapid action of all current drugs against chloroquine-resistant malaria and *Plasmodium falciparum*.

MATERIALS AND METHODS

Materials used

Eggs of *Gallus domesticus* (white leghorn chicken), candling box, antimalarial drug α/β arteether, disposable tuberculin syringes, distil water, incubator with humidifier, measuring

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tape, electronic weighing machine, Vernier caliper, and magnifying glass.

Methodology
Inclusion criteria
- Proper calcified eggs with the intact shell
- Eggs having air cell at the broader end
- Egg's having air cell without any blood clot.

Exclusion criteria
- Eggs with cracked shell due to improper calcification
- Eggs not having air cell at the broader end
- Eggs having a blood clot in air cell.

The present study was designed to assess the effects of α/β arteether, an antimalarial drug on developing chick embryo in 300 eggs of white leghorn chicken. The study was conducted in the Department of Anatomy, Santosh Medical College, Santosh Nagar, Ghaziabad U. P. in association with the Department of Anatomy, Gold Field Institute of Medical Sciences and Research, Chhainsa, Ballabgarh, Faridabad, Haryana. The eggs were obtained from Metro Feeds, Agro Products, Village Dabua, DabuaPali road, Faridabad.

The method was adopted as adopted by Natekar,[5] Singroha *et al.*[6] Thongphanich and Roongruangchai[7] and Hussein *et al.*[8] The eggs were candled to exclude defective ones and to mark outline the exact location of air cell with a pencil. For this purpose, a specially made wooden box was procured. This box had a connection for an electric bulb and was painted black from inside. The slots for the chicken eggs made on the top. The weight of selected eggs was found between 33.30 g to 48.15 g (average 40 g).

Before start of the study, 10 fertilized eggs of white leghorn chicken were developed in an incubator without normal saline or distilled water or any drug. On the 20th day of incubation eggs was sacrificed, and chick embryos were obtained. Their weight by electronic machine and CR length by digital Vernier's calipers were measured. The minimum weight of embryo 17.98 g, maximum weight 27.98 g, and minimum crown-rump length 4.90 cm. Maximum crown-rump length 6.70 cm were noted. The weight and crown-rump length of embryos same and between the minimum and maximum measurement were considered as normal [Table 1].

The selected eggs were thoroughly washed with soap water solution and put in an incubator with their broad end up where chorioallantoic membrane is situated. The temperature and humidity inside the incubator were maintained at 38°C and

80%, respectively, with no additional CO_2 or O_2. The eggs were tilted two times a day. On the 5th day of incubation, eggs were removed from the incubator and candled again before administration of the drug.

Drug-α/β arteether
- Availability-Injection E-Mal– α/β arteether 150 mg/2 ml
- Name of the manufacturer-Themis Medicare
- Recommended dose.
    - 150 mg by intramuscular route for 3 consecutive days (adults)
    - 3 mg/kg of body weight by intramuscular route for 3 consecutive days (children).

Dose titration
Antimalarial drug α/β arteether used in this study is available as an ingredient in commercial injection E-Mal product of Themis Medicare. Injection E-Mal contains α/β arteether as an active ingredient in the strength of 150 mg in 2 ml solution.

Dose titration as embryo weight with respect to human dose:
- The weight of chick embryo on the 5th day of incubation = 0.13 g (The Embryonic Development of chickens and humans. Kathryn Robinson on March 20, 2013. 208 Tweet.)
- Human dose of drug = 3 mg/kg (1000 g) of body weight
- The dose of the drug for chick embryo on the 5th day of incubation = 3 X.13/1000=0.00039 mg
- Injection E Mal 2 ml contains-150 mg of drug solution A
    - Step 1.1 ml of solution A was taken in a test tube

1 ml of solution A contains = 75 mg of drug.
- Step. 2 1 ml of solution A diluted with 9 ml distilled water to get 10 ml of solution B

1 ml of solution B contains = 7.5 mg of drug.
- Step. 3 1 ml of solution B diluted with 9 ml of distilled water to get 10 ml of solution C

1 ml of solution C contains = 0.75 mg of drug.
- Step. 4 1 ml of solution C diluted with 9 ml of distilled water to get 10 ml of solution D
- Step. 5 1 ml of solution D diluted with 9 ml of distilled water to get 10 ml of solution E.

1 ml of solution E contains = 0.075 mg of drug.
- Step. 6 1 ml of solution E diluted with 9 ml of distilled water to get 10 ml of solution F.

1 ml of solution F contains = 0.0075 mg of drug.

Feature	Chick number 1	Chick number 2	Chick number 3	Chick number 4	Chick number 5	Chick number 6	Chick number 7	Chick number 8	Chick number 9	Chick number 10	Mean	SD
Weight of embryo (g)	21.39	20.97	21.47	23.98	27.98 maximum	21.42	20.63	21.89	17.98 minimum	22.19	21.99	0.5656
CRL of embryo (cm)	6.24	6.27	5.98	5.77	6.7 maximum	4.90 minimum	6.07	6.05	5.99	6.52	6.04	0.1979

Table 1: Weight and crown rump length of chick embryos without any solution

SD: Standard deviation, CRL: Crown rump length

Hence, 0.00039 mg drug is present in 0.00039/0.0075 = 39/750 = 0.052 ml
- 1 ml syringe contains = 40 units
- 0.052 ml = 40 × 0.052 = 2.08 units
- 2 units contains = 0.00039 mg of drug
- 1 units contains = 0.00039 × 1/2 = 0.000195 mg. of drug
- 3 units contains = 0.00039 × 3/2 = 0.000585 mg. of drug
- 4 units contains = 0.00039 × 4/2 = 0.00078 mg. of drug
- 5 units contains = 0.00039 × 5/2 = 0.00097 mg. of drug
- 6 units contains = 0.00039 × 6/2 = 0.00117 mg. of drug
- 7 units contains = 0.00039 × 7/2 = 0.00136 mg. of drug.

Dose setting

Then, seven groups having 10 eggs each on the 5th day of incubation solution F was injected by insulin syringe in the dose of 1unit, 2 units, 3 units, 4 units, 5 units, 6 units, and 7 units. The 8th group also having 10 eggs was developed without any solution. The toxicity of the drug was estimated on the bases of the hatchability and development of chicks. At the dose of 0.00136 mg (7 units), chick were failed to hatch and the rate of mortality was 90% in contrast with smaller doses 2 units, 3 units. 4 units, 5 units and 6 units the survival rate was 50% to 70%. However, with still smaller dose 1 unit no apparent abnormality was detected. From the above study, it was proved that dose of 7 units (0.00136 mg) of the solution F is lethal dose (LD (50)) for chick embryo hence 5 doses between LD (50) (7 units = 0.00136 mg) and minimum dose (1 unit = 0.000195 mg) 2 units (0.00039 mg), 3 units (0.000585 mg), 4 units (0.00078 mg), 5 units (0.00097 mg), and 6 units (0.00117 mg) were selected for assessment.

Method for drug administration

Eggs were divided into five experimental groups A, B, C, D, E having 30 eggs each and five control group a, b, c, d, e for each test group, respectively, having 30 eggs each. Experimental groups A, B, C, D and E were exposed to α/β arteether with dose of 2 units (0.00039 mg), 3 units (0.000585 mg), 4 units (0.00078 mg), 5 units (0.00097 mg), and 6 units (0.00117 mg) and control groups were treated with the same volume of normal saline. The solution was taken in an insulin syringe. The broad end of eggs was wiped with sterile gauze pad moistened with 70% isopropyl alcohol solution. A hole was made in egg shells in the center of the surface over the air cell with a lancet. It was taken care not to damage the shell membranes with the point of the drill. The needle of the insulin syringe filled with the injectable solution was inserted horizontally into the air cell, and the solution was injected. The needle of the syringe was sterilized after wiping with sterile 70% isopropyl alcohol swab between each injection. The hole of the shell was sealed with candle melted wax immediately after injection. After the injection sealed eggs were kept again into an incubator at 38°C and 70% to 80% humidity and tilted two times a day. On the 20th day of incubation, eggs were removed from the incubator. The eggshells were broken, and the embryos were collected.

Staining of skeleton

Twentieth day old chick embryos were washed with clean tap water and eviscerated and processed through cartilage and bone double staining method described by McLeod[9] with little modifications. The embryos were fixed in absolute ethyl alcohol for 7 days. After fixation, embryos were stained for 4 days in a solution prepared by: (a) 1 volume of 0.1% Alizarin-red-s in absolute ethyl alcohol, (b) 1 volume of glacial acetic acid, and (c) 1 volume of absolute ethyl alcohol. For proper and uniform staining, 100 ml solution was used for each skeleton in a separate jar to avoid overlapping. After staining, the embryos were washed with tap water and placed in 2% KOH solution for 48 h for maceration. Macerated stained embryos were passed up through the increasing concentration of glycerin as 20%, 40%, 60%, 80%, and then ultimately stored in pure glycerin with some thymol crystals to avoid mold proliferation. Photographs were taken in photographic laboratory of the department.

OBSERVATIONS

A number of dead and alive embryos were noted and percentage of lethal effects given in Table 2 and Figures 1-3. Number of chick embryos with abnormal weight and crown rump length given in Table 3. Morphological and skeletal abnormalities were noted, analyzed and compared with control groups with necked eyes and magnifying glass.

Morphological malformations

No morphological malformation was observed in all control groups and experimental Groups A and B. The chick embryos 3.3% from , 23.3% from experimental Group D, 10% from control Group E, and 20% from experimental Group E were found suffering from scanty feathers [Figure 4]. Subcutaneous hemorrhage (hematoma) was found in chick embryos obtained 3.3% from experimental Group C, 10% from experimental Group D, and 33.3% from experimental Group E [Figures 5 and 6]. Nearly 3.3% chick embryos from experimental Group C, 6.6%

Table 2: Lethal effects induced by α/β artether in developing chick embryo

Name of the groups	Number of fertile eggs used	Number of dead embryos	Number of live embryos	Percentile lethality (%)
a	30	0	30	0
A	30	2	28	6.6
b	30	1	29	3.3
B	30	2	28	6.6
c	30	2	28	6.6
C	30	5	25	16.6
d	30	1	29	3.3
D	30	8	22	26.6
e	30	1	29	3.3
E	30	13	17	43.3

Name of group	Number of chicken born with normal weight	Number of chicken born with less than normal weigh	Number of chicken born with more than normal weigh	Number of chicken born with normal CRL	Number of chicken born with less than normal CRL	Number of chicken born with more than normal CRL
a	27	2	1	28	1	1
A	23	5	2	28	1	1
b	28	2	0	28	2	0
B	22	6	2	23	6	1
c	26	1	3	26	4	0
C	19	8	3	17	9	4
d	27	2	1	25	4	1
D	17	11	2	17	12	1
E	28	2	0	23	5	2
E	15	15	0	13	17	0

Table 3: Abnormal weight and crown rump length due to effects of α/β artether

CRL: Crown rump length

Figure 1: Normal chick embryo

Figure 2: Growth retarded and dead chick embryo

Figure 3: Growth retarded dead chick embryo with failure to retraction to yolk sac

Figure 4: Alive chick embryo with scanty feathers and failure to retraction to yolk sec

from experimental Group D, 6.6% from control Group E, and 26.6% from experimental Group E were found fail to retract of their yolk sacs [Table 4].

Skeletal abnormalities

The axial and appendicular skeletal abnormalities are given in Tables 5-18 and Figures 7-19.

Table 4: Gross anatomical malformations due to effects of α/β arteether

Abnormalities	Groups									
	a	A	B	B	c	C	d	D	E	E
Macrocephay (head enlargement)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Microcephaly (short head)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1
Exophthalmia (bulging eye)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Microphthalmia (short eye)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Narrow neck	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Twisted neck	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Long beak	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Short beak	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Subcutaneous hemorrhage (hematoma)	Nil	Nil	Nil	Nil	Nil	1	Nil	3	Nil	10
Limbus deformity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Ectopic viscerum	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Scanty feathers	Nil	Nil	Nil	Nil	Nil	Nil	1	7	3	7
Failure to retraction of yolk sac	Nil	Nil	Nil	Nil	Nil	1	Nil	2	2	8

Table 5: Skeletal abnormalities in skull due to effects of α/β arteether

Groups	Retarded ossification (%)	Short maxilla	Short mandible (%)	Congenital absence of cranium	Any other abnormality
a	Nil	Nil	Nil	Nil	Nil
A	4 (13.3)	Nil	Nil	Nil	Nil
b	Nil	Nil	Nil	Nil	Nil
B	6 (20)	Nil	Nil	Nil	Nil
c	1 (3.3)	Nil	Nil	Nil	Nil
C	8 (26.6)	Nil	Nil	Nil	Nil
d	1 (3.3)	Nil	Nil	Nil	Nil
D	8 (26.6)	Nil	Nil	Nil	No
e	1 (3.3)	Nil	Nil	Nil	No
E	12 (40)	Nil	2 (6.66)	Nil	No

Table 6: Skeletal abnormalities in vertebral column due to effects of α/β arteether

Groups	Not/poorly ossified (%)	Absent	Displaced or fused (%)	Scoliosis (%)	Lordosis (%)	Kyphosis	Absent or kinky tail	Any other abnormality
A	Nil	Nil	Nil	1 (3.3)	Nil	Nil	Nil	Nil
A	1 (3.3)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
B	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
B	4 (13.3)	Nil	Nil	Nil	1 (3.3)	Nil	Nil	Nil
C	1 (3.3)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
C	6 (20)	Nil	Nil	1 (3.3)	1 (3.3)	Nil	Nil	Nil
D	1 (3.3)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
D	6 (20)	Nil	1 (3.3)	2 (6.6)	2 (6.6)	1 (3.3)	Nil	Nil
E	3 (10)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
E	10 (33.3)	Nil	1 (3.3)	2 (6.6)	2 (6.6)	2 (6.6)	Nil	Nil

DISCUSSION

Teratogenic mechanism covers the series of events that occur from the movement a teratogen exerts its influence on the tissues of developing chick embryos to the final results of malformations either structural or functional. The initial event in teratogenesis may be brought about by a teratogen by its own or by its metabolites and the influence being exerted (a) on an organ primordium which will be malformed later or (b) on the embryonic tissues other than the one going to be malformed, or (c) the maternal tissue or placenta. A review of teratological studies shows that a large number of factors initiate the abnormal development at cellular or subcellular levels such as genetic mutation, chromosomal aberration, mitotic interference, altered nucleic acid integrity or function, lack of precursors, and substrates altered energy sources, enzyme inhibition, fluid osmolytic imbalance, and changed membrane characteristics.[10] Morphogenesis and tissue interaction[11]

Figure 5: Hematoma at right lateral side of the neck

Figure 6: Hematoma at ventral abdominal wall

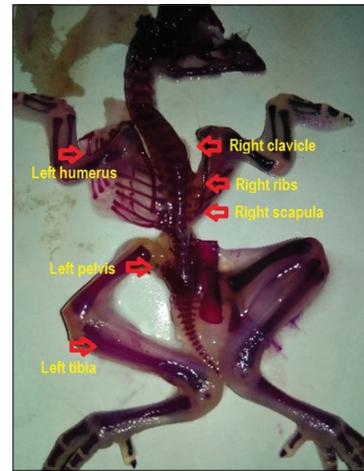
Figure 7: Normal skeleton of chick embryo

Figure 8: Poor ossification of pelvis, humerus, and tibia of left side and ribs clavicle and scapula of left side

Figure 9: Poorly ossified and thin femur and poorly ossified tibia of the right side and under developed ribs and clavicle of left side
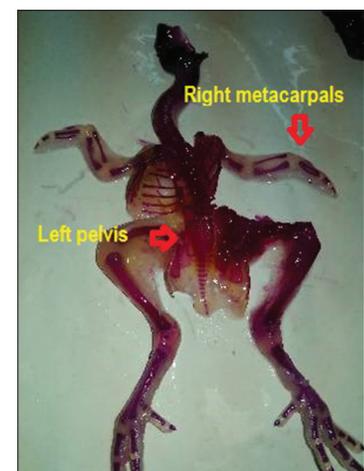

Figure 10: Poor ossification of metacarpals of the right side and pelvis of left side

may take place between two or more tissues of different properties (heterotype) or between like cells (homotype),[12] and these are concerned as the central control mechanism for differentiation and morphogenesis.

Many cytotoxic agents at lower doses inhibit proliferative activity and result in reduced cell number, which play a central role in most malformations and teratogenesis. Sequential

Table 7: Abnormalities in rib due to effects of α/β arteether

Groups	Incomplete or poorly ossified (%)	Absent or displaced (%)	Synostosis (%)	Any other abnormality
a	1 (3.3)	Nil	Nil	Nil
A	3 (10)	1 (3.3)	Nil	Nil
b	1 (3.3)	Nil	Nil	Nil
B	2 (6.6)	2 (6.6)	Nil	Nil
c	1 (3.3)	Nil	Nil	Nil
C	4 (13.3)	3 (10)	Nil	Nil
d	2 (6.6)	Nil	Nil	Nil
D	6 (20)	3 (10)	Nil	Nil
e	2 (6.6)	1 (3.3)	Nil	Nil
E	11 (36.6)	2 (6.6)	1 (3.3)	Nil

Table 8: Sternal abnormalities due to effects of α/β arteether

Groups	Poorly or not ossified (%)	Displaced (%)	Dumb bell shaped (%)	Synostosis	Asternia	Any other abnormality
a	Nil	Nil	Nil	Nil	Nil	Nil
A	2 (6.6)	1 (3.3)	Nil	Nil	Nil	Nil
b	Nil	Nil	Nil	Nil	Nil	Nil
B	3 (10)	Nil	1 (2)	Nil	Nil	Nil
c	1 (3.3)	Nil	1 (10)	Nil	Nil	Nil
C	4 (13.3)	1 (3.3)	Nil	Nil	Nil	Nil
d	Nil	Nil	1 (2)	Nil	Nil	Nil
D	8 (26.6)	2 (6.6)	2 (4)	Nil	Nil	Nil
e	1 (3.3)	Nil	1 (10)	Nil	Nil	Nil
E	12 (40)	1 (3.3)	2 (4)	Nil	Nil	Nil

Table 9: Abnormal changes in skeleton of upper limb exposed to α/β arteether (Test group A in comparison to Control group a)

Name of bone	Control group a				Test group A			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened	Bent or displacement	Any other change
Clavicle	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Scapula	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Humerus	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Radius	Nil	Nil	Nil	Nil	1 (3.3)	Nil	Nil	Nil
Ulna	Nil	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil
Carpals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metacarpals	1 (3.3)	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil
Phalanges	Nil	Nil	Nil	Nil	5 (16.6)	Nil	Nil	Nil

Table 10: Abnormal changes in skeleton of upper limb exposed to α/β arteether (Test group B in comparison to Control group b)

Name of bone	Control group b				Test group B			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Clavicle	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Scapula	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Humerus	Nil	Nil	Nil	Nil	1 (3.3)	1 (3.3)	Nil	Nil
Radius	Nil	Nil	Nil	Nil	1 (3.3)	Nil	1 (3.3)	Nil
Ulna	Nil	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil
Carpals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metacarpals	Nil	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil
Phalanges	1 (3.3)	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil

Table 11: Abnormal changes in skeleton of upper limb exposed to α/β arteether (Test group C in comparison to Control group c)

Name of bone	Control group c				Test group C			
	Poorly ossified or not ossified	Shortened	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change
Clavicle	Nil	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil
Scapula	Nil	Nil	Nil	Nil	1 (3.3)	Nil	Nil	Nil
Humerus	Nil	Nil	Nil	Nil	2 (6.6)	Nil	2 (6.6)	Nil
Radius	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Ulna	Nil	Nil	Nil	Nil	3 (10)	Nil	Nil	Nil
Carpals	Nil	Nil	Nil	Nil	1 (3.3)	Nil	Nil	Nil
Metacarpals	Nil	Nil	Nil	Nil	2 (6.6)	Nil	2 (6.6)	Nil
Phalanges	Nil	Nil	Nil	Nil	2 (6.6)	Nil	Nil	Nil

Table 12: Abnormal changes in skeleton of upper limb exposed to α/β arteether (Test group D in comparison to Control group d)

Name of bone	Control group d				Test group D			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change
Clavicle	1 (3.3)	Nil	Nil	Nil	3 (10)	Nil	1 (3.3)	Nil
Scapula	1 (3.3)	Nil	Nil	Nil	2 (6.6)	Nil	1 (3.3)	Nil
Humerus	1 (3.3)	Nil	Nil	Nil	4 (13.3)	Nil	Nil	Nil
Radius	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Ulna	1 (3.3)	Nil	Nil	Nil	6 (20)	Nil	Nil	Nil
Carpals	No.	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metacarpals	2 (6.6)	Nil	Nil	Nil	6 (22)	Nil	2 (6.6)	Nil
Phalanges	1 (3.3)	Nil	Nil	Nil	7 (23.3)	Nil	2 (6.6)	Nil

Table 13: Abnormal changes in skeleton of upper limb exposed to α/β arteether (Test group E in comparison to Control group e)

Name of bone	Control group e				Test group E			
	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Clavicle	Nil	Nil	Nil	Nil	4 (13.3)	Nil	2 (6.6)	Nil
Scapula	Nil	Nil	Nil	Nil	3 (10)	Nil	2 (6.6)	Nil
Humerus	1 (3.3)	No	Nil	Nil	4 (13.3)	Nil	Nil	Nil
Radius	Nil	Nil	Nil	Nil	4 (13.3)	2 (6.6)	Nil	Nil
Ulna	1 (3.3)	Nil	Nil	Nil	6 (20)	2 (6.6)	Nil	Nil
Carpals	1 (3.3)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metacarpals	Nil	1 (3.3)	Nil	Nil	6 (20)	Nil	3 (10)	Nil
Phalanges	Nil	Nil	Nil	Nil	6 (20)	4 (13.3)	4 (1.33)	Nil

Table 14: Abnormal changes in skeleton of lower limb exposed to α/β arteether (Test group A in comparison to Control group a)

Name of bone	Control group a				Test group A			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Pelvis	Nil	Nil	Nil	Nil	3 (10)	Nil	Nil	Nil
Femur	Nil	Nil	Nil	Nil	3 (10)	1 (3.3)	Nil	Nil
Tibia	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fibula	Nil	Nil	Nil	Nil	2 (6.6)	Nil	2 (6.6)	Nil
Tarsals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metatarsals	1 (3.3)	Nil	1 (3.3)	Nil	4 (13.3)	Nil	2 (6.6)	Nil
Toes	1 (3.3)	Nil	1 (3.3)	Nil	4 (13.3)	Nil	Nil	Nil

Table 15: Abnormal changes in skeleton of lower limb exposed to α/β arteether (Test group B in comparison to Control group b)

Name of bone	Control group b				Test group B			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Pelvis	1 (3.3)	Nil	Nil	Nil	2 (6.6)	1 (3.3)	Nil	Nil
Femur	Nil	Nil	Nil	Nil	2 (6.6)	Nil	2 (6.6%)	Thinning
Tibia	Nil	Nil	Nil	Nil	3 (10)	Nil	1 (3.3)	Thinning
Fibula	1 (3.3)	Nil	Nil	Nil	4 (13.3)	4 (13.3)	1 (3.3)	Nil
Tarsals	No	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metatarsals	1 (3.3)	Nil	Nil	Nil	4 (13.3)	Nil	2 (6.6)	Nil
Toes	1 (3.3)	Nil	Nil	Nil	6 (20)	Nil	4 (13.3)	Nil

Table 16: Abnormal changes in skeleton of lower limb exposed to α/β arteether (Test group C in comparison to Control group c)

Name of bone	Control group c				Test group C			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change
Pelvis	Nil	Nil	Nil	Nil	5 (16.6)	Nil	1 (3.3)	Nil
Femur	Nil	Nil	Nil	Nil	4 (13.3)	Nil	1 (3.3)	Nil
Tibia	1 (3.3)	Nil	1 (3.3)	Nil	5 (16.6)	Nil	1 (3.3)	Thinning
Fibula	Nil	Nil	Nil	Nil	3 (10)	Nil	2 (6.6)	Nil
Tarsals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Metatarsals	Nil	Nil	Nil	Nil	6 (20)	1 (3.3)	2 (6.6)	Nil
Toes	Nil	Nil	Nil	Nil	6 (20)	2 (6.6)	2 (6.6)	Nil

Table 17: Abnormal changes in skeleton of lower limb exposed to α/β arteether (Test group D in comparison to Control group d)

Name of bone	Control group d				Test group D			
	Poorly ossified or not ossified (%)	Shortened	Bent or displacement (%)	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Pelvis	1 (3.3)	Nil	Nil	Nil	6 (20)	1 (3.3)	2 (6.6)	Thinning
Femur	Nil	Nil	Nil	Nil	3 (10)	1 (3.3)	1 (3.3)	Nil
Tibia	Nil	Nil	1 (3.3)	Nil	Nil	Nil	2 (6.6)	Thinning
Fibula	Nil	Nil	Nil	Nil	5 (16.6)	1 (3.3)	2 (6.6)	Nil
Tarsals	Nil	Nil	Nil	Nil	Nil	Nil	No	Nil
Metatarsals	1 (3.3)	Nil	Nil	Nil	5 (16.6)	1 (3.3)	4 (13.3)	Nil
Phalanges	1 (3.3)	Nil	Nil	Nil	4 (13.3)	2 (6.6)	3 (10)	Nil

Table 18: Abnormal changes in skeleton of lower limb exposed to α/β arteether (Test group E in comparison to Control group e)

Name of bone	Control group e				Test group E			
	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change	Poorly ossified or not ossified (%)	Shortened (%)	Bent or displacement (%)	Any other change
Pelvis	Nil	1 (3.3)	1 (3.3)	Nil	8 (26.6)	3 (10)	4 (13.3)	Thinning
Femur	Nil	1 (3.3)	Nil	Nil	7 (23.3)	4 (13.3)	4 (13.3)	Thinning
Tibia	Nil	Nil	Nil	Nil	7 (23.3)	3 (10)	3 (10)	Nil
Fibula	1 (3.3)	Nil	Nil	Nil	6 (20)	4 (13.3)	2 (6.6)	Nil
Tarsals	Nil	Nil	Nil	Nil	No	Nil	Nil	Nil
Metatarsals	Nil	Nil	Nil	Nil	8 (26.6)	Nil	2 (6.6)	Nil
Toes	Nil	Nil	Nil	Nil	9 (30)	Nil	1 (3.3)	Nil

events in this process are inhibition of RNA synthesis and reduction of mucopolysaccharides, hydroxyproline,[13] and phospholipid contents in the palatal shelves.[14] Reduced proliferative rates has resulted in a lot of studies with
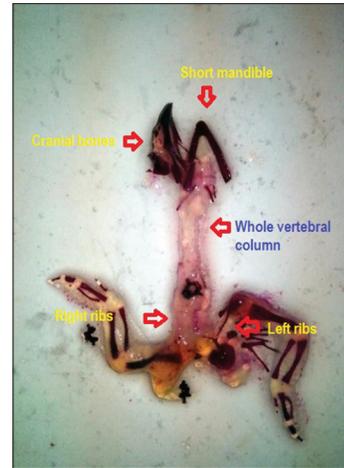
Figure 11: Poor ossification of cervical vertebrae

Figure 12: Short mandible, poorly developed skull bones, vertebral column, and ribs of both sides and undeveloped pelvic limbs

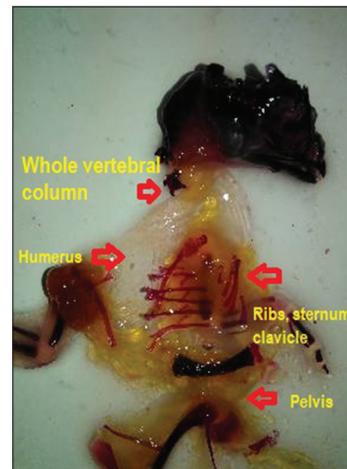**Figure 13:** Kyphosis, poorly ossified ribs of the right side and femur and tibia of left side

Figure 14: Poorly developed vertebral column humerus of the right side and right pelvic limb with poorly ossified and displaced ribs of both sides

Figure 15: Poorly developed ribs of right side and pelvis, femur, and tibia of left side

Figure 16: Scoliosis with undeveloped left pelvis

cyclophosphamide,[15] hypervitaminosisA, aminocentesis,[15] and chlorpromazine.[16]

Multiple teratological investigations reveal that physical or chemical insult produces cell necrosis in tissues destined to
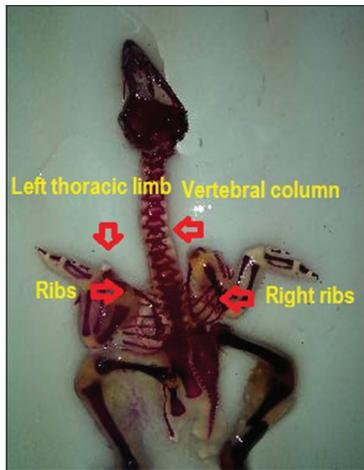
Figure 17: Poor ossification of whole vertebral column, bilateral ribs, and whole left thoracic limb

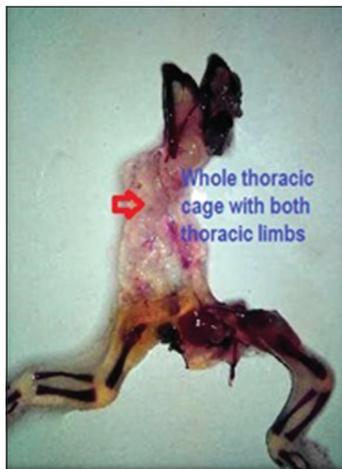**Figure 18:** Undeveloped pelvis and poor ossification of femur and tibia of left side

Figure 19: Undeveloped B/L thoracic limb, B/L ribs and whole vertebral column

Figure 20: Percentile lethality in chick embryos exposed to α/β arteether

be malformed within a few hours or days.[17] It has found that higher doses cause cell death and teratogenesis and at lower kill the cell at a lower rate but may not produce malformations. There are varied responses to cytotoxic agents from cell to cell. This might be due to many reasons. Most important is intrinsic cell difference which determines whether the cell will die or not. With 6 aminonicotinomide, a severe vacuolation was found in ectodermal cells but not in mesodermal or endodermal derivatives.[18] The sensitivity of the cells depend on the nutritional state, which is carried out by diffusion. Cells farthest from nutritional source are affected most. There are abundant evidences that insecticides produce their acute toxic actions by inhibiting acetylcholinesterase.[19]

In the present study, α/β arteether in different doses effects embryonic chick development as indicated by the conditions such as lethality rate [Figure 20], growth retardation, and poor ossification of bones. Hence, many defects indicate the probable involvement of more than one biochemical lesion or mode of action. The observation on poor ossification of
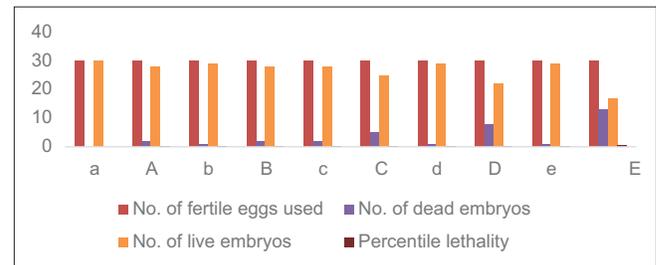
skeleton could be due to the impairment of chondrochondroitin sulfate, possibly through interference with an NAD-dependent process.[20] The inhibition of yolk sac membrane K-phase activity has been directly correlated with the lowering of the embryo NAD level and teratogenic signs.[21] This may be result of nutritional deficiencies, due to the failure of the embryo to receive some esters-metabolized products from the yolk sac.[22] The vertebral defects (lordosis) are attributable to acetylcholinesterase inhibition[23-26] and associated disruption of the cholinergic system.[27-29] α/β arteether has a long plasma life and narrow safety range. Therefore, adding the drug halfway through the treatment only add to the adverse effects and not to the therapeutic benefit. It should not misuse the newer antimalarial drug α/β arteether and need to preserve it for future. Research into α/β arteether is scanty and the parasite is fast developing resistance even for α/β arteether. Depletion of α/β arteether by misusing it may have nothing left for treating all drug-resistant malaria in nearly coming future. Therefore, α/β arteether, the drug of choice for the treatment for chloroquine-resistant malaria and life-threatening malaria falciparum should be used only when definitely indicated and not discriminately.α/β arteether should be used only when parasite index or other methods prove drug-resistant malaria. In addition, α/β arteether can be used in cases of hyperparasitemia or life-threatening complications on account of their ability to clear the parasitemia to other antimalarial drugs. Infection with plasmodium falciparum and chloroquine resistant plasmodium

vivax is dangerous so effective and safe treatment is required. At present, α/β arteether is drug of choice for the treatment of malaria falciparum and chloroquine resistant malaria vivax because no other drug is available for the same. Due to the unavailability of any other drug and more overdue to insufficient qualified health infrastructure in hugely populated India α/β arteether and other drugs of its group are being using very frequently resultant α/β arteether resistance malaria cases are started to report. The role of α/β arteether is controversial in many previous studies. In a study α/β arteether and other drugs of its group were found embryo-fetal and angiogenesis inhibitor in rats.[10] Using artemisinin combination therapy during the 1st trimester in 685 pregnant Senegal found 2.6% stillbirth, 2.8% miscarriages, and in 1.6% major congenital malformations were identified.[11] In previous animal experiments, there are clear evidence of death of embryos and some evidence of gross anatomical abnormalities[9] by using α/β arteether and drugs of its group.

There are so many research fellows[30,31] who worked on teratogenic effects of α/β arteether and other drugs of its group with respect to pregnancy and concluded toxic in early pregnancy and safe in the 2nd and 3rd trimester but as far as teratogenic effects of higher dose of the same drug is concern the data are very much insufficient almost negligible. In the present study, the increased mortality and poor ossification of the skeleton in experimental groups are significant and directly or indirectly implies the growth of the embryos.

Conclusion

The present study revealed increased mortality percentage, growth retardation, poor ossification and alteration in the curvature of vertebral column, etc., due to effects of the α/β arteether. The drug is toxic, especially when used in a higher dose and for a long period. At present, there is no alternative drug available for the treatment of chloroquine-resistant malaria and malaria falciparum except α/β arteether. Therefore, α/β arteether and other artemisinins should be used only after the establishment of proper diagnosis in recommended dose only not in a higher dose and not for a long duration.

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Conflicts of interest
There are no conflicts of interest.

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