Folic Acid Effect on Artemether-Induced Cardiac Anomalies in Mice Embryos
Muhammad Shahid Akhtar¹ and Mohammad Tahir²

ABSTRACT
Objective: To determine the role of folic acid in preventing the adverse effects of artemether, on fetal heart in Albino mice.
Study Design: Experimental study.
Place and Duration of Study: Department of Anatomy, University of Health Sciences, Lahore, from July 2011 to January 2012.
Methodology: Eighteen pregnant Albino mice were randomly divided into three groups A, B, and C of six mice each. The control group A was treated with intramuscular injection of solvent arachis oil 10.7 mg/kg, group B was given intramuscular injection of artemether 10.7 mg/kg, and group C was given intramuscular injection of artemether 10.7 mg/kg and folic acid 4.93 mg/kg, dissolved in 0.1 ml of distilled water orally, from 6th to 10th day of pregnancy. On 18th gestational day, the mice were sacrificed, and dissected to deliver live fetuses of group A (n=46), group B (n=16), and group C (n=20) were delivered. After dissection of the fetuses, the hearts were isolated and fixed in 10% formalin and processed in a usual way for histological examination with the light microscope after H&E staining, using X4, X10, and X40 objectives. The sections were evaluated for presence of septal defects, thickness of ventricular and atrial septa was calculated by micrometry. For statistical analysis, SPSS version 18 was used.
Results: Post-Hoc Tukey’s test indicated statistically significant difference in mean atrial septal thickness between groups A and B (p < 0.001), groups B and C (p=0.015), and insignificant difference between groups A and C (p=0.178). There was statistically significant thinning of ventricular septa between groups A and B (p < 0.001), groups B and C (p < 0.001), and groups A and C (p < 0.001).
Conclusion: Folic acid protected the toxic effects of artemether on the development of heart.
Key Words: Artemether. Atrial septal thickness. Ventricular septal thickness.

INTRODUCTION
Folic acid, a component of vitamin B complex, is required for DNA and RNA synthesis. It promotes erythropoiesis and is used commercially in food supplements.¹ It cannot be synthesized by the body and only 10 percent or less of folic acid in active form is present in the normal diet.² The risk of congenital malformations, like cardiac malformations induced by retinoic acid (Vitamin A), craniofacial deformities, neonatal mortality from neural tube defects, imperforate anus and cleft lip and palate are reduced by periconceptional use of folic acid.³-⁶
Artemether is a new antimalarial agent. Its mechanism of action against malaria is not well understood. It is suggested that production free oxygen radicals are responsible for killing the malaria parasite.⁷
Intramuscular artemether appears to be an excellent alternative to intravenous quinine as resistance to quinine is common in Asia.⁸ Pregnant women are highly susceptible to malaria infestation. Malaria during pregnancy causes severe maternal complications like abortion, premature labour and still-births, which are higher in plasmodium falciparum infestation.⁹ Severe maternal anemia due to malaria is a leading cause of maternal mortality.¹⁰,¹¹ Artemether has been shown to counter the complications of malaria in pregnant women.⁸ Various studies in mice, rats, and rodents showed that when artemether and other derivatives of artemisinin were given orally or by injection, during vulnerable period of organogenesis, caused death of the embryo, blood vessels anomalies, ventricular septal defects, malformed ribs, shortened or bent long bones, defects in scapulae and incompletely ossified pelvic bones.⁹,¹⁰,¹² These embryotoxic effects were due to destruction of primitive erythroblasts, which are present early in the developing embryo, by artemether and other derivatives of artemisinin, resulting in transient deficiency of the primitive erythroblasts.¹³ Artemether and other derivatives of artemisinin are not recommended in the first trimester of pregnancy due to limited safety data on its use in human, although these can be given during second and third trimesters.
It has been reported that artemether caused miscarriage and cardiac malformations like ventricular septal defect when given during vulnerable period of gestation in animals, whereas protective role of folic acid on cardiac malformations induced by it has not been previously investigated. The present study was, therefore, designed to investigate if there is a protective role of folic acid on

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the adverse effects of artemether on development of heart in Albino mice.

**METHODOLOGY**

It was a randomized controlled experimental study conducted at the Department of Anatomy, University of Health Sciences, Lahore. Twenty-four adult BALB/c strain Albino mice, eighteen females (75%), and six males (25%), 6 - 8 weeks old, weighing from 30 to 35 grams were kept under control conditions of temperature 23 ±2°C, humidity 50 ±5% with regular 12-hour light/dark cycles. Male and female mice were put together in a ratio of 1:3 in a single cage for mating. Females were examined early morning everyday for the presence of vaginal plug; its presence indicated that mating had occurred and the day was considered as day 0 of gestation. Pregnant mice were separated, housed in a separate cage and randomly divided into three groups, A, B and C, having six female mice each. Commercially available preparations of artemether, folic acid 97% and arachis oil, the solvent for preparation of artemether injection were used. Group A was treated with single intramuscular injection of solvent, arachis oil 10.7 mg/kg, Group B was given artemether, 10.7 mg/kg once daily by intramuscular injection, and Group C was treated with artemether, 10.7 mg/kg by intramuscular injection and folic acid 4.93 mg/kg in 0.1 ml. of distilled water once daily, from 6th to 10th day of pregnancy.

The pregnant mice of group A, B and C were sacrificed on the 18th gestational day to deliver the fetuses. All live male and female fetuses were included in the study. The live fetuses of group A (n= 46), group B (n=16) and group C (n=20) were then euthanized with chloroform, examined for gross malformations under dissecting stereo microscope and fixed in 10% formalin solution for 72 hours after decapitation. The thoracic cavity of the fetuses was opened by midline thoracoabdominal incision; heart was identified and observed under dissecting microscope for its position and that of the great vessels and any visible gross anomalies. The heart was carefully dissected and removed with the root of great vessels for histological examination. The heart was isolated immediately after the animal was sacrificed and rapidly washed with distilled water to clear its blood contaminants and fixed in 10% formalin for 48 hours. The fixed complete fetal hearts were processed in automatic tissue processor. The tissue blocks were made, sections were cut at 5µm thickness and mounted on the albumenized glass slides, which were allowed to dry on a slide warmer. These sections were stained with Hematoxylin and Eosin (H & E) for histological study. The stained sections were studied under light microscope using X4, X10, and X40 objectives. These sections were evaluated for presence of the atrial and ventricular septal defects. The thickness of the atrial and ventricular septa was calculated by micrometry. After calibrating the ocular with the stage micrometer, one division of eye-piece micrometer was calculated equal to 27.27 µm. The scale of the eyepiece micrometer was superimposed on the ventricular septum of the fetal heart. The number of divisions of eye piece micrometer covering the ventricular septum was calculated, multiplied by 27.27, which was taken as the actual thickness of the ventricular septum in microns. Then the scale of the eyepiece micrometer was superimposed on the cross section of the atrial septum of the fetal heart. The number of divisions, covering the atrial septum multiplied by 27.27, gave the actual thickness of the atrial septum in microns. The thickness was measured at 3 different sites and mean was taken as final value.

The collected information of the study groups was analyzed using Statistical Package for Social Sciences (SPSS) version 18. The difference in the quantitative measurement was tested by one way ANOVA. Post-Hoc Tukey’s test was applied to identify the difference in group mean values. Relevant descriptive statistics was mean and standard deviations for quantitative variables. The p-value of ≤ 0.05 was considered statistically significant.

**RESULTS**

Mean atrial septal thickness of fetuses heart was 107.30 ±18.04, 84.08 ±11.25 and 99.53 ±14.59 µm in groups A, B and C, respectively. One way ANOVA showed statistically significant difference in the mean atrial septal thickness when compared among groups (p-value < 0.0001; Table I).

Post-Hoc Tukey’s test indicated statistically significant difference in mean atrial septal thickness between groups A and B (p-value < 0.0001) and groups B and C (p-value = 0.015). Statistically insignificant difference in atrial septal thickness was observed between groups A and C (p-value = 0.178; Table II).

Mean ventricular septal thickness was 593.41 ±37.19, 434.61 ±42.27 and 536.76 ±35.69 µm in groups A, B, and C, respectively. One way ANOVA showed statistically significant difference in the mean ventricular septal thickness when compared among groups (p-value < 0.0001; Table I).

Post-Hoc Tukey’s test indicated statistically significant difference in mean ventricular septal thickness between groups A and B (p < 0.0001), groups B and C (p < 0.0001).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial septal thickness (µm)</td>
<td>107.30 ±18.04</td>
<td>84.08 ±11.25</td>
<td>99.53 ±14.59</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Ventricular septal thickness (µm)</td>
<td>593.41 ±37.19</td>
<td>434.61 ±42.27</td>
<td>536.76 ±35.69</td>
<td>&lt; 0.0001*</td>
</tr>
</tbody>
</table>

p-value ≤ 0.05 is statistically significant.
and groups A and C (p < 0.0001) showing significant decreased in ventricular septal thickness in groups B and C (Table III).

**DISCUSSION**

Macroscopic examination of hearts did not reveal any structural difference between the control and those of the experimental groups in this study. The position of heart, lungs and diaphragm was normal in fetuses from the control and treated groups. Gross examination of the heart revealed no structural malformation. Atrial septum thickness reduction was statistically significant in group B when compared to group A, and not significant when compared between groups C and A (Table II). Ventricular septal thickness was reduced in groups B and C and was statistically significant when compared to group A (Table III). There were statistically significant decrease in atrial septum thickness and ventricular septal thickness in group B when compared with group C.

Cardiovascular system is first to develop and is essential for providing nutrients to the developing embryo. In mouse, it begins on the 7th day of gestation. Ventricular septal defects and various defects of skeleton, like malformed ribs, cleft sternebrae, shortened or bent long bones were seen in pregnant rats orally administered with artesunate, a derivative of artemisinin, at doses of 6, 10 and 16.7 mg/kg, once daily, starting from day 6 of gestation for 12 days throughout, the period of organogenesis. In rabbits, however, doses of 5, 7 and 12 mg/kg once daily, starting from day 6 of gestation for 13 days produced comparable results. There was an increased incidence of anomalies, when a single oral dose of 17 mg/kg of artesunate was given on day 10 of gestation to rats; in rabbits the embryolethal effect was observed as abortions and total loss of litter. These developmental effects were seen without any evidence of maternal toxicity.

It has been observed that abnormalities of heart appeared in the rat embryo, after a single oral administration of 17 mg/kg of artesunate on day 10 of gestation; these changes are manifested in the form of reduced thickness of ventricular and atrial walls and cardiac cavity due to thin trabeculae carneae which became more evident over the next few days. The heart showed signs of recovery in the rat embryo that survived to day 14 of gestation, but its development got retarded.

There is strong evidence supporting the conclusion that periconceptional multivitamin supplementation containing folic acid may reduce the risk of congenital heart defects. The Hungarian randomized clinical trials in 1984-1991 have demonstrated that the risk for congenital anomalies of cardiovascular system and urinary system was reduced significantly after the periconceptional multivitamin supplementation containing folic acid.

**CONCLUSION**

The current study investigated the protective role of folic acid on the adverse effects of artemether on the developing heart of the mouse. The thicknesses of atrial and ventricular septa were significantly decreased in artemether treated group. The statistically significant amelioration in atrial and ventricular septa was observed in folic acid treated group and the values of the parameters were nearly comparable to those in the control group, which was an evident indication of protective effect of folic acid on heart.

**REFERENCES**


Table II: Post-Hoc Tukey’s test shows multiple comparison of atrial septal thickness of heart of fetuses in µm among groups.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>(i) Group of animals</th>
<th>(j) Group of animals</th>
<th>Mean Difference (i-j)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial septum thickness</td>
<td>A dimension3</td>
<td>B</td>
<td>23.21902</td>
<td>4.68567</td>
<td>.000</td>
<td>12.0265 - 34.4116</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>7.76602</td>
<td>4.32407</td>
<td>.178</td>
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<tr>
<td></td>
<td>B dimension3</td>
<td>A</td>
<td>-23.21902</td>
<td>4.8567</td>
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<td>-34.4116 - 12.0265</td>
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<tr>
<td></td>
<td></td>
<td>C</td>
<td>-15.45300</td>
<td>5.41491</td>
<td>.015</td>
<td>-28.3875 - 2.5185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>15.45300</td>
<td>5.41491</td>
<td>.015</td>
<td>2.5185 - 28.3875</td>
</tr>
</tbody>
</table>

* p value ≤ 0.05 is statistically significant.

Table III: Post-Hoc Tukey’s test shows multiple comparison of ventricular septal thickness of heart of fetuses in µm among groups.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>(i) Group of animals</th>
<th>(j) Group of animals</th>
<th>Mean Difference (i-j)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
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<tr>
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<td>56.65441</td>
<td>.000</td>
<td>132.5528 - 185.0538</td>
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<tr>
<td></td>
<td></td>
<td>C</td>
<td>-102.14887</td>
<td>12.69989</td>
<td>.000</td>
<td>-132.4848 - 71.8130</td>
</tr>
<tr>
<td></td>
<td>B dimension3</td>
<td>A</td>
<td>-158.80329</td>
<td>10.98957</td>
<td>.000</td>
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<tr>
<td></td>
<td></td>
<td>C</td>
<td>-102.14887</td>
<td>12.69989</td>
<td>.000</td>
<td>-132.4848 - 71.8130</td>
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<tr>
<td></td>
<td>C dimension3</td>
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<td></td>
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<td>12.69989</td>
<td>.000</td>
<td>71.8130 - 132.4848</td>
</tr>
</tbody>
</table>

* p value ≤ 0.05 is statistically significant.

3. Awan UN. Prevention of vitamin A induced teratogenic effects on heart by folic acid in albino mice [thesis]. University of Health Sciences, Lahore. 2008.


