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Histopathological Effect Of Artesunate In Chicks Liver Subjected To Exposure To Artesunate At Embryonic Stage

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ABSTRACT

Introduction: Artesunate is obtained from the Artemisia plant, scientifically referred to as Qinghaosu, and is indigenous to China. Artemisinin derivatives are a specific group of drugs that have the highest effectiveness in treating Chloroquine resistant Plasmodium vivax and Plasmodium falciparum malaria compared to all other antimalarial drugs available. Malaria imposes a substantial socio-economic burden on the population of India, experiences approximately two million cases of malaria each year, leading to a substantial number of deaths. The chick embryo is commonly used as an animal model to investigate the harmful effects of various drugs in research. The objective of this study is to assess the histopathological effects of artesunate on the liver of developing chick embryos.

Material & Methods: A total of 165 eggs of Gallus domesticus were used for this study. On the 5th day, eggs were injected with artesunate and normal saline as per dose titration, divided into four experimental groups and control groups. Further incubation and manual hatching were done on 18th day. Liver was isolated from the embryo for histological slide preparation; further analysis of each slide was done under a binocular microscope to determine the histopathological changes and abnormalities.

Results: Based on histopathological analysis, we observed the abnormalities of fat deposition and fatty changes, sinusoids with mild to severe lymphocyte infiltration, sinusoidal dilation, sinusoidal congestion, extensive inflammatory changes, and liver degeneration.

Conclusion: The recommended dose of artesunate has a mild hepatotoxic effect but teratogenic risk increases with an increase in drug dose in the liver.

Keywords: *Artesunate, Hepatotoxicity, Teratogenic effects, Gallus Domesticus*

1. INTRODUCTION

Among seven infectious diseases, namely malaria, diarrhoea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia, malaria accounts for 85% of the socio-economic burden on human beings worldwide.[1,2] According to data published by the World Health Organization (WHO) in 2018, malaria caused over 219 million illnesses and 435,000 deaths in 2017. India accounts for 4% of all malaria cases, whereas Southeast Asia accounts for 87%.[3] In 2017, Chhattisgarh, Jharkhand, Madhya Pradesh, and Odisha accounted for about 74.1% of all malaria cases in India, with Odisha having the highest percentage at 40%. In 2017, 194 deaths and 0.84 million cases of malaria were reported by the National Vector Borne Disease Control Program (NVBDCP).[4] Based on the World Health Organization's (WHO) Global Technical Strategy for Malaria, the Indian government launched a campaign in 2016 to eradicate malaria from the country by 2030. [5, 6]

Artesunate antimalarial drugs are the first choice for treating and curing chloroquine-resistant malaria and falciparum-sensitive malaria. [7] In 1967, the Chinese government discovered that medicinal herbs known as qinghao or qinghaosu possess antimalarial and antipyretic properties.[8] Artesunate is a semi-synthetic, water-soluble antimalarial drug that is a derivative of the drug artemisinin. Artesunate comprises the sodium succinyl salt of dihydroartemisinin.[9] Compared to other antimalarial drugs, artesunate plays a significant role against chloroquine-resistant Plasmodium vivax and Plasmodium falciparum malaria. [10] Various studies on artesunate drugs have demonstrated toxic effects, and common side effects include embryotoxicity, genotoxicity, neurotoxicity, cardiotoxicity, and allergic reactions.[11] hepatotoxicity (tissue degeneration, sinusoidal congestion, and inflammatory cell infiltration was seen in a study done on wistar rats liver).[12, 13]

The chick embryo is an appropriate model for embryological study due to its availability throughout year and large size of chicken eggs, as well as the easy artificial incubation and hatching under controlled humidity and temperature conditions. Furthermore, the chick embryo's general embryological development and body structure are comparable to humans. [14]

In chicks' digestive system, the liver is the largest accessory gland and lies caudal and anterior to the lungs and heart, respectively. Microscopically, Chick's liver shows hexagonal hepatic lobules, and each lobule has a hepatic portal vein, proper hepatic artery, bile duct, and central vein. [15] The liver plays an important role in both the storage and metabolic activities of the drugs. [13, 16] This study aims to evaluate the histopathological impact of artesunate on chicks' livers exposed to it at the embryonic stage.

2. MATERIAL AND METHODS

For this study, we collected 165 Gallus domesticus (white leghorn chicken) eggs.[17] Prior to experimentation, we sanitised all the eggs. We measured the egg crown rump length and weight using digital verniercaliper and Kerro electronic scales respectively. The inclusion criteria included properly calcified eggs with intact shells, air cells at the broader end, and air cells without blood clots, while the exclusion criteria excluded cracked shells, air cell non-existence and blood clots. On the 5th day, each egg was viewed in a wooden electric candle box to mark air cell space in egg shells and a small hole was made using needle in egg shells over air cell space. Artesunate drug was injected through air cell holes using a ROMO JET brand (1ml - disposable) syringe following dose titration. We divided the eggs into four experimental groups (A, B, C, D) tested them with artesunate drugs and four control group (a, b, c, d) tested with normal saline respectively. Each group consists of 20 eggs, namely group A tested with 0.0003 mg (0.3 ml), B with 0.0005 mg (0.5 ml), C with 0.0007 mg (0.7 ml), and D with 0.0009 mg (0.9 ml) and same unit of normal saline group. A total of five eggs were devoid of any drug treatment in this study. After injecting the solution eggs air shells holes were sealed with the wax, marking was done for numbering, units, and drug name on the shell side, and the eggs were placed in an incubator with an average temperature of 38 °C and a humidity of 53-63%. The experimental eggs were rotated 2-3 times per day till 18th day of incubation. In this study, we manually hatched the eggs on the 18th day and isolated the liver of a chick embryo by dissecting anterior end of body cavity. Histological tissue processing and the slide preparation of isolated liver were done by using automatic tissue processor (LEICA TP 1020) and rotary microtome (LEICA RM2255). Haematoxylin – Eosin stain (Qualigens) were used for liver tissue staining. The study of each slide was performed under a binocular microscope to identify histopathological. changes and abnormalities in the chicks' liver at embryonic stage.

Statistical analysis

Exploratory descriptive statistical analysis was done using microsoft excel and the SPSS-25 software.

3. RESULTS

The study analysed the liver of chick embryos exposed to artesunate drugs in experimental group "A," revealing that one egg did not develop into a chick embryo out of 20. Eight livers showed histopathological changes. [Table No.01, Chart No.01] Three chick embryo livers showed fat deposition and fatty changes with a proportion mean of 0.15 and S.D. 0.36635 , while eight livers had sinusoids with mild lymphocyte infiltration, with a proportion mean of 0.4 and S.D. 0.50262. [Chart No.02, Fig. No.02]

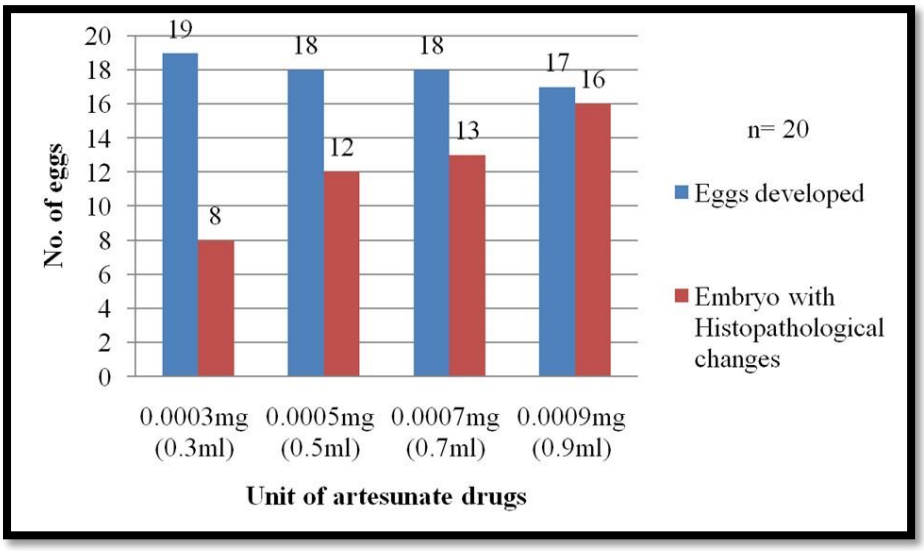
The experimental group "B" found that two out of 20 eggs did not develop into chick embryos, and twelve chick embryo livers showed histopathological changes. [Table No.01, Chart No.01] Twelve chick embryo livers showed moderate fat deposition and fatty changes, with a proportion mean of 0.6 and a standard deviation of 0.50262, while ten livers had sinusoids with mild lymphocyte infiltration, with a proportion mean of 0.50.4 and a S.D. of 0.51299. [Chart No.02, Fig. No.03]

Experimental group "C" revealed that out of 20 eggs, two did not develop into chick embryos, and 13 livers showed histopathological changes, while five lacked such changes. [Table No.01, Chart No.01] Twelve chick embryos liver having fat deposition and fatty changes with a mean of 0.6 and S.D. 0.50262; Thirteen liver having sinusoids with moderate lymphocyte infiltration with a proportion mean of 0.65 and S.D. 0.48936, Nine chick embryos having sinusoidal dilation with a proportion mean of 0.45 and S.D. 0.51042; and Two chick embryos having liver degeneration and necrosis with a proportion mean of 0.1 and S.D. 0.30779. [Chart No.02, Fig. No.04]

Table No. 01: Artesunate Experimental Group (A, B, C & D)

Name of Group	Drugs in Unit	No. Of Eggs	No. of eggs develop in to Chick embryo	No. of eggs not develop in to chick embryo	Embryo with Histopathological changes	Embryo with no Histopathological changes
A	0.0003mg (0.3ml)	20	19	1	8	11
B	0.0005mg (0.5ml)	20	18	2	12	6
C	0.0007mg (0.7ml)	20	18	2	13	5
D	0.0009mg (0.9ml)	20	17	3	16	1

Chart No. 01: Artesunate Experimental Group (A, B, C & D)



The experimental group "D" found that out of 20 eggs, 03 did not develop into chick embryos, and 16 chick embryo livers showed histopathological changes, with one exhibiting no changes. [Table No.01, Chart No.01] It reveals that 16 chick embryo livers show fat deposition and fatty changes, sinusoids with severe lymphocyte infiltration with a proportion mean of 0.8 and S.D. 0.41039, two chick embryos having sinusoidal dilation with a proportion mean of 0.1 and S.D. 0.30779, three chick embryos having sinusoidal congestion with a proportion mean of 0.15 and S.D. 0.36635, thirteen having extensive inflammatory changes with a proportion mean of 0.65 and S.D. 0.48936, and thirteen chick embryos having liver degeneration and necrosis with a proportion mean of 0.65 and S.D. 0.48936. [Chart No.02, Fig. No.05,06]

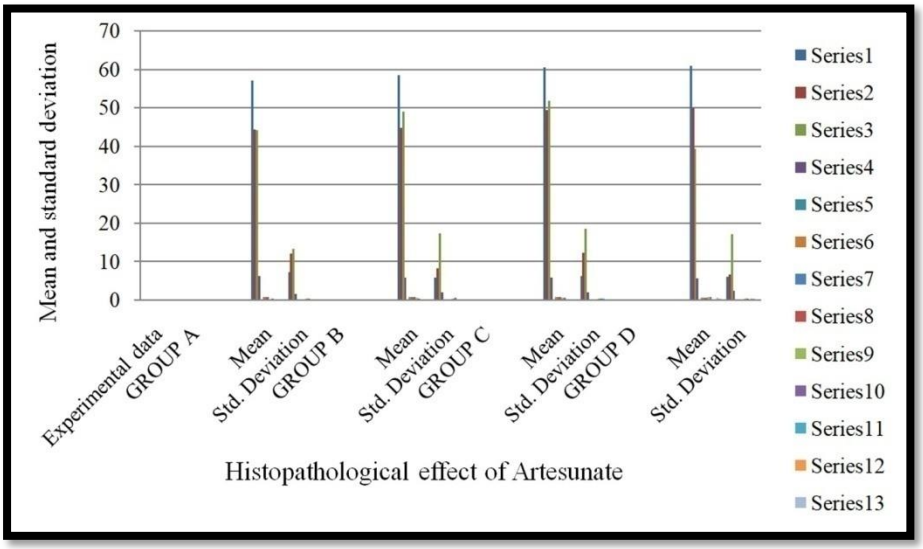
The study found that control groups a, b, c, and d had no or mild changes in the hepatocyte, sinusoid, and central vein, resulting in no histopathological changes in the liver of a chick embryo, and only one egg did not develop into a chick embryo. [Table no.02, Fig. No.01, Chart No.02] 05 eggs without drugs and normal saline showed normal hepatocytes, portal vein, hepatic artery, bile duct, and central vein.

Table No. 02: Normal Saline Control Group (a, b, c & d)

Name of Group	Normal Saline	No. Of Eggs	No. of eggs develop in to Chick embryo	No. of eggs not develop in to chick embryo	Embryo with Histopathological changes	Embryo with no Histopathological changes
a	0.3ml	20	20	0	0	20

b	0.5ml	20	20	0	0	20
c	0.7ml	20	20	0	0	20
d	0.9ml	20	19	1	0	19

Chart No. 02. Histopathological effect of artesunate in chicks liver at embryonic stage showing Series 1 indicate eggs weight in gram, Series 2 indicate eggs crown rump length in mm, Series 3 indicate embryo weight on 18th days ,Series 4 indicate embryo CRL on 18th days, Series 5 indicate number of dead embryos, Series 6 indicate normal hepatocytes, Series 7 indicate normal sinusoids, Series 8 indicate normal central vein, Series 9 indicate fat deposition and fatty changes, Series 10 indicate sinusoids with lymphocyte infiltration, Series 11 indicate sinusoidal dilation, Series 12 indicate sinusoidal congestion, Series 13 indicate extensive inflammatory changes. and Series 14 indicate liver degeneration and necrosis



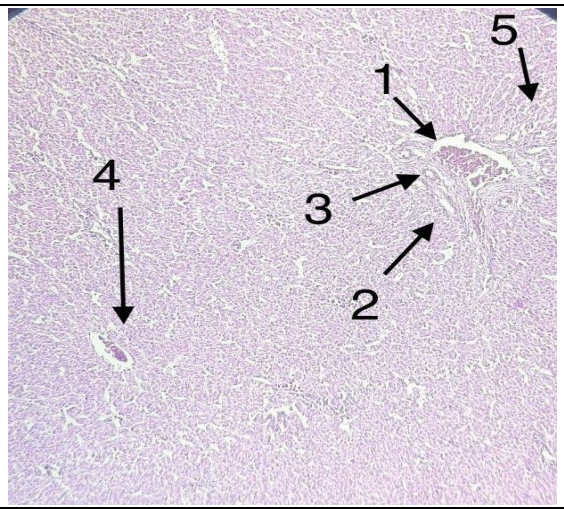


Fig. No.01 Normal saline-0.7 ml, showing 1. Portal vein, 2. Hepatic artery 3. Bile duct 4. Central vein 5. Hepatocyte (H&E, 10X) and scale bare 100µm

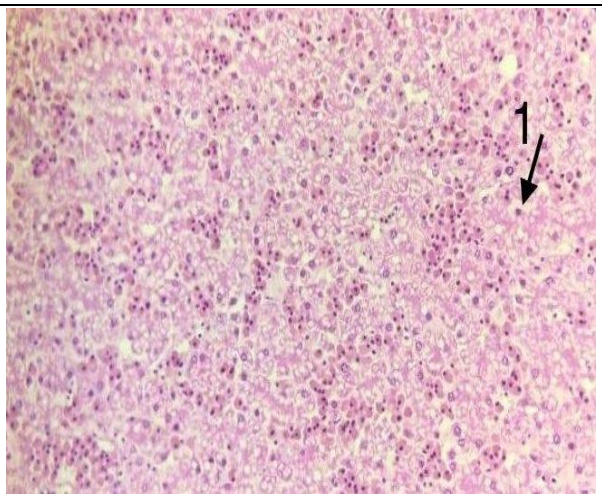


Fig. No.02 Artesunate (0.3ml) showing with 1. Mild lymphocyte infiltration (H&E, 40X) and scale bare 40µm

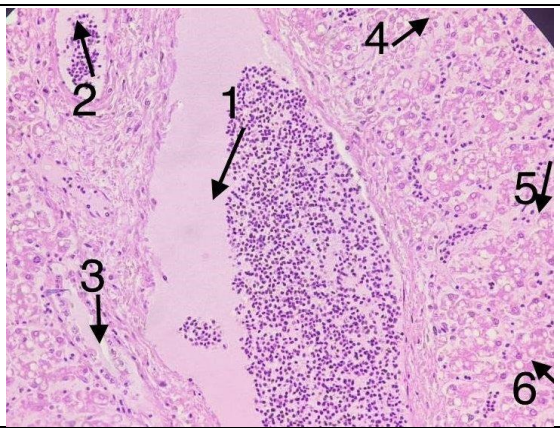


Fig. No.03 Artesunate (0.5 ml) showing 1. Portal Vein, 2. Hepatic Artery, 3. Bile Duct, 4. Hepatocyte, 5. Sinusoids with Moderate Lymphocyte Infiltration and 6. Mild Adipose Tissue (H&E, 40X) and scale bare 40µm

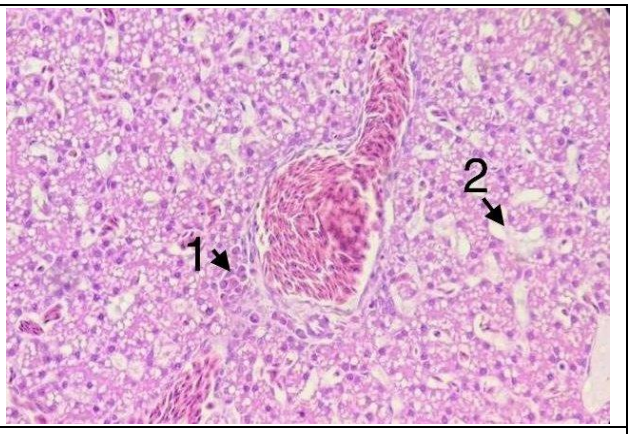


Fig. No.04 Artesunate (0.7ml) showing 1. lymphocyte infiltration, plasma cell, neutophils around central vein and fatty changes, 2. Hepatic sinusoidal dilatation (H&E, 40X) and scale bare 40µm

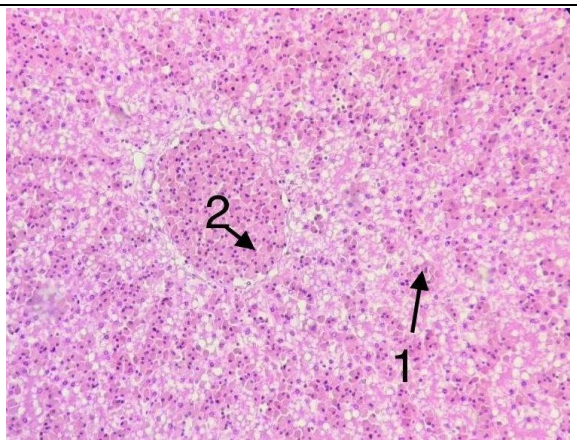


Fig. No.05 Artesunate (0.9ml) 1. Severe congestion and hepatocyte degeneration 2. Completely filled central vein (H&E,40X) and scale bare 40µm

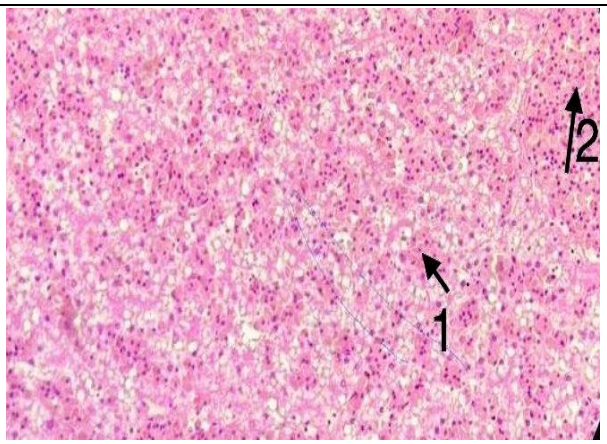


Fig. No.06 Artesunate (0.9ml) 1. Severe congestion and hepatocyte degeneration, 2. Central vein (H&E, 40X)

4. DISCUSSIONS

Plasmodium parasites cause malaria, a life-threatening disease that poses a significant threat in Africa and Asia. Many antimalarial drugs, reported to cause mild to harmful effects on the body, skeleton, and organs, are in use. [18] The World Health Organization introduced Artesunate as the first-line treatment to combat multi-drug-resistant Plasmodium falciparum malaria. [19] The study investigated the histopathological impact of artesunate on the liver of chicks exposed to it. The experimental group "A" showed mild fat deposition, fatty changes, sinusoids, and lymphocyte infiltration in chick livers with minimal effect on tissue architecture, consistent with a study by Felix Monday Onyijeetal.(2012). [12]

The experimental group "B" showed moderate fat deposition and fatty changes, sinusoids, and mild lymphocyte infiltration after being administered 0.0005 mg (0.5 ml), and the experimental group "C" was administered 0.0007 mg (0.7 ml) of a drug, resulting in fat deposition, sinusoids with lymphocyte infiltration, sinusoidal dilation, liver degeneration, and necrosis, which correlate with the study done by A.M. Izunya et al. (2010). [13]

In our study, the higher dose of artesunate (0.0009 mg/0.9 ml) revealed marked changes in fat deposition and fatty changes, sinusoids with severe infiltration of lymphocytes, sinusoidal congestion, extensive inflammatory changes, and liver degeneration with necrosis. These findings corroborate with the study carried out on mammals by Olurishe et al. (2011) [20] and Nwanjo et al. (2007). [21]

The study by Felix Monday et al. (2012) and Izunya AM (2010) comparable with our finding that control groups a, b, c, and d, administered normal saline, showed no or mild changes in the hepatocyte, sinusoid, and central vein, indicating no

histopathological changes. [12,13]

5. LIMITATIONS OF THE STUDY

The animal model used in this study was the chick embryo (avian); however, human subjects are necessary to have a better knowledge of the effects of artesunate on humans.

6. CONCLUSION

In this study, we found that artesunate had mild to severe effects on the chick's liver, causing abnormalities such as fat deposition, fatty changes, lymphocyte infiltration, sinusoidal dilation, congestion, extensive inflammatory changes, and liver degeneration, as observed through histopathological analysis. The recommended dosage has a less or no hepatotoxic effect, and the effects increase as the drug dose is increased.

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Conflicts of interest: There are no conflicts of interest.

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