ejpmr, 2017,4(2), 240-246

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FETAL HEPATORENAL TOXICITY OF ARTEMETHER/LUMEFANTRINE (COARTEM®) IN SECOND TRIMESTER OF PREGNANCY IN ALBINO RATS

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Article Received on 14/12/2016 **Article Revised on 03/01/2017** **Article Accepted on 25/01/2017**

ABSTRACT

The effect of artemether/lumefantrine (Coartem®) administration in second trimester of pregnancy on the morphometric indices of rat fetuses, fetal liver and kidney as well as amniotic alpha fetoprotein was investigated in this study. Twenty (20) pregnant albino Wistar rats were divided into 4 groups of 5 animals per group and used for the study. Group 1 served as control and received no drug treatment. Group 2 received 8mg/kg (therapeutic dose) of coartem while Groups 3 and 4 received 16mg/kg and 24mg/kg of coartem respectively. The drug was administered twice daily for three days being its normal therapeutic regimen. The animals were sacrificed on the 20th day of pregnancy under chloroform anaesthesia, fetuses delivered through uterotomy and assessed for morphological deformities. Fetal liver and kidney were harvested and evaluated for histopathology. Amniotic fluid was obtained and used to assay for alpha fetoprotein (AFP) levels. A significant ($P < 0.05$) dose dependent decrease in fetal body weight, crown rump length was observed in the fetuses of the treated groups when compared to the control. Significant decrease in amniotic alpha fetoprotein was also observed in Group 2 and 4 while the AFP level in Group 3 was significantly increased ($P < 0.05$). The histology of fetal liver revealed mild to severe alterations in cellular cytoarchitecture and features such as hepatocytic necrosis, dilated central veins and sinusoidal spaces were observed in the treatment groups. Sections of the fetal kidney showed atrophic and degenerated glomeruli as well as tubular necrosis whose severity increased with increasing dose of coartem. The study has shown that artemether/lumefantrine is toxic to fetal liver and kidney when administered to pregnant albino wistar rats in second trimester and fetal growth was also reduced significantly.

KEYWORDS: Artemether/Lumefantrine, Coartem, Fetal hepatorenal toxicity, pregnancy.

INTRODUCTION

Clinical trials of drugs are often carried out without including pregnant subject because of concerns over the safety of the mother and the developing fetus[1]. Consequently, most drugs are marketed with limited information on their safety during pregnancy and therefore are not recommended for use by pregnant women, yet some of the drugs are still widely used by pregnant women. Furthermore, the drugs are often unavoidably used in chronic disease or acute diseases that can harm the mother and the unborn child if left untreated. There are limited safety data in pregnancy for drugs targeting tropical disease, as these are not widely used in the countries with more robust pharmacovigilance system and anti-malarial drugs are good examples[2]. Malaria can have a devastating effect on both the mother and the fetus[3,4], and pregnant women require prompt treatment with safe and effective anti-malarial drugs when infected[5].

The artemisinins are the most effective and rapidly acting anti-malarials to date, providing lifesaving benefits to children, adults and pregnant women[6]. The limited information about their safety is reassuring and the World Health Organisation now recommends the use of artemisinin-based combination therapies (ACTs) in the second and third trimester of pregnancy as uncertainty remains about their safety in early pregnancy[5-8]. However, in an attempt to generate more information on the safety of anti-malarial drugs in pregnancy, international anti-malarial pregnancy exposure registry has been established in malaria endemic regions as a cost effective approach[5]. ACTs readily available include artemether/lumefantrine, artesunate/amodiaquin, artesunate/mefloquin, artesunate/pyrimethamine/sulfadoxine, dihydroartemisinin/piperaquin. Artemether and lumefantrine combination is the most widely used of these ACTs.

Artemether is an artemisinin derivative while lumefantrine is a racemic mixture of a synthetic flourent derivative often in a fixed combination of 20mg artemether and 120 mg lumefantrine per tablet. Both drugs are active against blood schizonts of the malaria parasite and have met WHO pre-qualification criteria of efficacy, safety and quality[9,10]. Artemether and lumefantrine combination is indicated for treatment of children and infants with acute, uncomplicated infections due to *Plasmodium falciparium* or mixed infections. It is also indicated for treatment of drug resistant malaria infections acquired in areas where parasites may be resistant to other anti-malarial drugs[11,12].

Artemisinin and its derivatives are toxic to malaria parasites in-vitro at nanomolar concentration whereas micromolar concentration is required for toxicity to mammalian cells[13]. Artemisinin, also known as qinghaosu, is a sesquiterpene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood). It's been in use for over a thousand years in China and is observed to be potent and rapidly acting against blood schizontocide and is active against all *Plasmodium* species with unusual broad activity against asexual parasites and gametocytes. Artemisinin and its derivatives inhibit an essential calcium adenosine triphosphatase, pfATPase 6[14]. Artemisinin is a potent inducer of its own metabolism, inducing the cytochrome P450 enzyme CYP2B6 to catalyse its conversion to an inactive metabolite[15]. Artemisinins have been reported to be safe and well tolerated. Adverse reactions include gastrointestinal disturbances, dizziness, tinnitus, neutropenia, elevated liver enzyme values and electrocardiographic abnormalities. Type 1 hypersensitivity reactions are the only reported serious adverse effect while neurotoxicity have also been reported in animal models at particularly high dose but has not yet been substantiated in humans[16-20]. Lumefantrine belongs to the aryl amino alcohol groups of anti-malarials which also include quinine, mefloquine and halofantrine. Its mechanism of action involves heme detoxication. Lumefatrine is often co-formulated with artemeter and is active against multidrug resistance *P. falciparum*. Reports are available stating that lumefantrine has no significant toxicities; however, mild side effects include nausea, abdominal discomfort and dizziness[21].

Due to potential embryotoxicity of artemisinins identified in animal studies, artemisinins are not considered safe for use in first trimester of pregnancy[22]. Studies have reported that artemether and lumefantrine in pregnancy shows reduced plasma concentrarion[23]. However, there is dearth of information on the effect of administration of artemether/lumefantrine during second trimester of pregnancy on fetal liver and kidney of albino wistar rats. This study is designed to evaluate the effect of administration of artemether/lumefantrine (Coartem®) in second trimester of pregnancy on the morphometric indices of rat fetuses, fetal liver and kidney as well as amniotic alpha fetoprotein.

MATERIALS AND METHODOLOGY

Drugs

Oral artemether/lumefantrine (Coartem) was manufactured by Norvartis International; one dispensable tablet contains 20mg of artemether and 120mg of lumefantrin.

Animals and Experimental design

Twenty (20) Female Wistar rats weighing between 180 – 200g were used for the study. They were obtained from the Animal House of the College of Health Sciences, University of Uyo, Nigeria. The animals were acclimatised for two weeks prior to the commencement of administration of the drugs. During this period, they were kept in well ventilated cages and were fed with rat pellets and water *ad libitum* throughout the experiment.

The animals were divided into 4 groups of 5 animals per group. In each groups, animals in their pro-estrous cycle were transferred into a designated cage overnight with matured sexually active male rats. The vaginal smears were taken following overnight mating and evaluated for the presence of spermatozoa. The presence of spermatozoa was taken to mark day zero of pregnancy.

The gestation period of albino Wistar rats is 21 days hence the administration of the drugs commenced on the 8th day (beginning of second trimester). The oral tablets of coartem were crushed into powder and stock solution prepared daily before administration. The administration was based on the design below:

Group 1: Served as control, received no treatment
Group 2: Received 8mg of coartem per kilogram body weight twice daily for three days
Group 3: Received 16mg of coartem per kilogram body weight twice daily for three days
Group 4: Received 24mg of coartem per kilogram body weight twice daily for three days

Collection of sample

The female rats were sacrificed on the 20th day of the pregnancy under chloroform anaesthesia. The fetuses were delivered by uterotomy, examined for abnormal external features and weighed. Liver and kidney tissues from the fetuses were harvested and preserved in 10% buffered formalin for histological evaluation. Amniotic fluid was collected for assay of alpha fetoprotein whose quantitative test kit is based on the principle of solid phase enzyme-linked immunosorbent assay.

Tissue processing for histology

Organ sections were passed through the processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining with haematoxylin and eosin (H and E) for examination under a light microscope. Photomicrographs of the tissue sections were taken using

a digital camera fitted to a light microscope at a magnification of 400X.

Statistical analysis

Statistical analysis was performed using analysis of variance and student's t-test. Experimental data was presented as mean ± standard error of mean (SEM). Values of ($p < 0.05$) were considered to be statistically significant.

RESULTS

Morphometric study

The rat fetuses in Groups 2, 3 and 4 whose mothers were treated with coartem in increasing doses of 8mg/kg, 16mg/kg and 24mg/kg respectively showed significant reduction in the weight and crown rump length of the fetuses when compared to rats in Group 1 (Controls). The decreases in body weight and crown rump length (CRL) of the fetuses were observed to be dose dependent. The result of the effect of artemether/lumefantrine on fetal body weight and CRL is presented in Table 1.

Table 1: Fetal Weight and CRL Following Administration of Artemether/Lumefantrine to Pregnant Albino Wistar Rats in Second Trimester.

Groups	Fetal weight (g)	Crown Rump Length (cm)
1 (control)	5.15 ± 0.24	4.74 ± 0.03
2 (8mg/kg)	4.36 ± 0.11*	4.40 ± 0.06
3 (16mg/kg)	3.60 ± 0.07*	4.21 ± 0.03*
4 (24mg/kg)	3.40 ± 0.04*	3.91 ± 0.10*

* Significantly different at $p < 0.05$.

Table 2 shows the value of alpha fetoprotein (AFP) expressed as mean ± standard error of mean. At 0.05 confident levels, there was a significant decrease of AFP concentration in all the treated groups when compared to the control except for Group 3 which received 16mg of coartem per kilogram body weight of rat.

Table 2: Amniotic Alpha Fetoprotein Following Administration of Artemether/Lumefantrine to Pregnant Albino Wistar Rats in Second Trimester

Groups	Amniotic Alpha Fetoprotein (ng/ml)
1 (control)	76.32 ± 0.41
2 (8mg/kg)	47.29 ± 1.03*
3 (16mg/kg)	89.63 ± 0.60*
4 (24mg/kg)	49.16 ± 0.44*

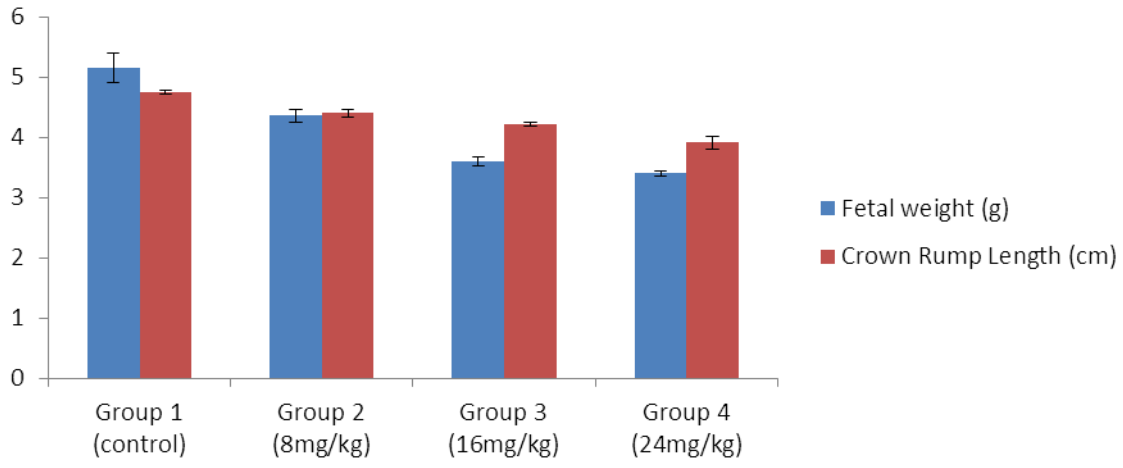

Figure 1: Fetal Body Weight and CRL Following Administration of Artemether/Lumefantrine to Pregnant Albino Wistar Rats in Second Trimester.

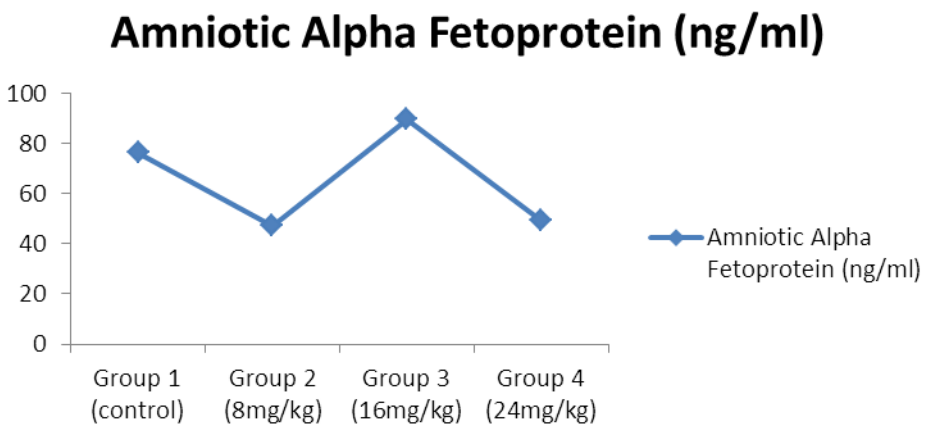

Figure 2: Amniotic Alpha Fetoprotein Following Administration of Artemether/Lumefantrine to Pregnant Albino Wistar Rats in Second Trimester

Effects of Artemether/Lumefantrine (Coartem) on the Histology of the Liver of Rats Fetuses

There is no alteration in the cytoarchitecture of the liver of feuses in Group 1 (Control). The central veins and the sinusoids were prominent while the hepatocytes had distinct outline (Plate A1). Fetal liver in Group 2 treated with 8mg/kg body weight of coartem showed dilated central veins and sinusoidal spaces as well as multinucleated hepatocytes with mild alteration in hepatocytic architecture (Plate A2). In Group 3 (treated with 16mg/kg of coartem), extensive hepatocytic necrosis and marked inflammatory cells were observed. The sinusoids were also dilated (Plate A3). Administration of 24mg/kg of coartem (Group 4) to pregnant rats showed fetal liver that distorted cytoarchitecture when compared to the control. There was marked mononuclear cells infiltrate and the central veins and the sinusoidal spaces were dilated (Plate A4).

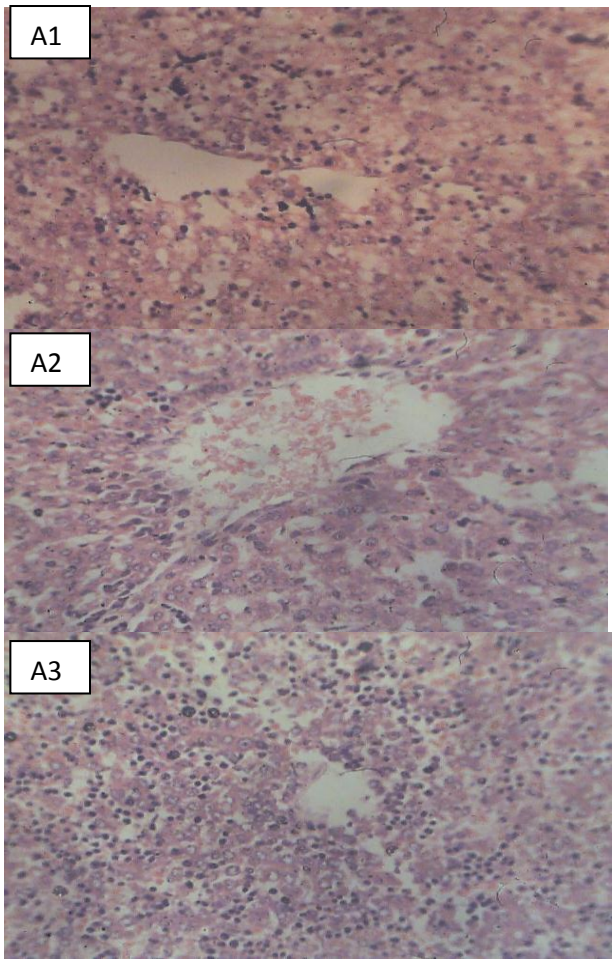

Plate A1: Photomicrograph of fetal liver from Group 1 (control) showing normal central vein, sinusoidal space and hepatocyte.

Plate A2: Photomicrograph of fetal liver from Group B whose mother received 8mg/kg of coartem showing congested and dilated central veins, congested and dilated sinusoidal space, multinucleated hepatocyte and alteration in hepatocytic architecture.

Plate A3: Photomicrograph of fetal liver from Group B whose mother received 16mg/kg of coartem showing effaced architecture with extensive hepatocyte necrosis and marked inflammatory cells with dilated sinusoids

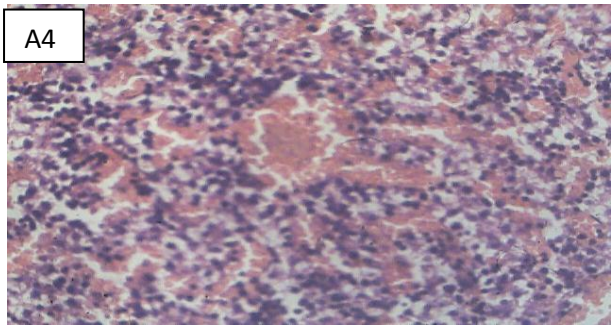

Plate A4: Photomicrograph of fetal liver from Group B whose mother received 24mg/kg of coartem showing distorted cytoarchitecture with marked mononuclear cell infiltrate and congested and dilated central vein

Effects of Artemether/Lumefantrine (Coartem) on the Histology of the Kidney of Rats Fetuses

The sections of fetal kidney from control (Group 1) showed distinct cortical medullary region. The cortex consists of numerous glomeruli with well outlined Bowman's capsule. The medullar consist of renal tubules of various sizes and shapes (Plate B1). Sections of fetal kidney from Group 2 (Plate B2) showed glomeruli degeneration and narrowing of the capsular spaces compared to the control. The Group treated with 16mg/kg of coartem (Group 3) revealed section of fetal kidney with atrophic and degenerated glomeruli as well as acute tubular necrosis (Plate B3). The fetal kidney from pregnant rats treated with 24mg/kg of coartem (Group 4) showed atrophic and severely degenerated glomeruli and acute tubular necrosis with moderate interstitial haemorrhagic haze.

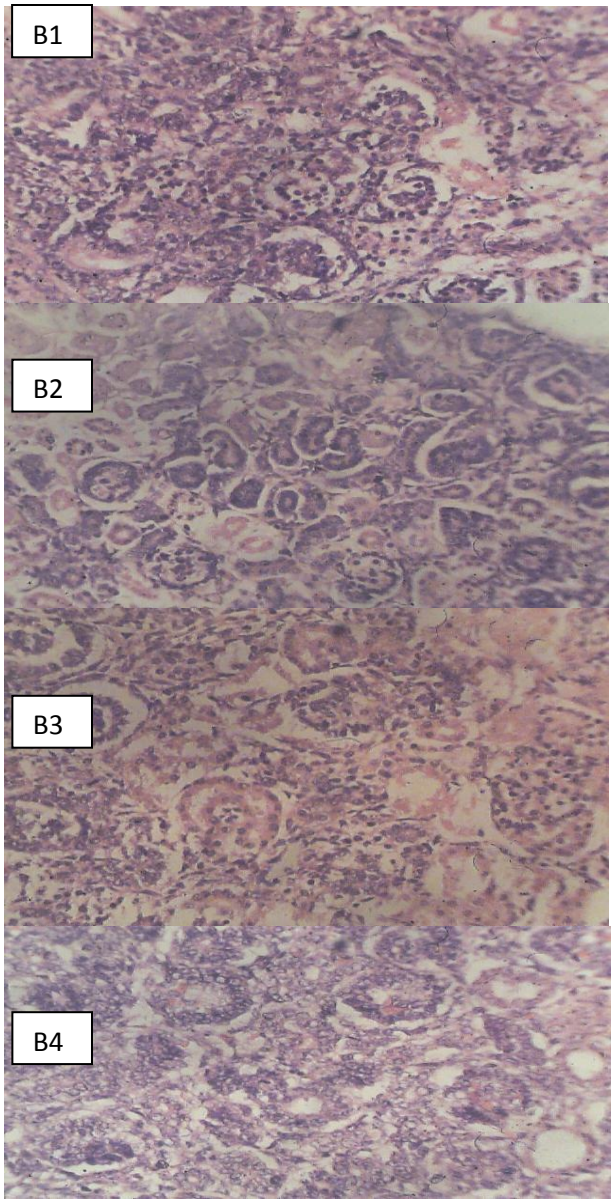

Plate B1: Photomicrograph of fetal kidney of Group 1 (control) showing normal tubules, glomeruli and Bowman's capsule.

Plate B2: Photomicrograph of fetal kidney of Group 2 whose mother received 8mg/kg of coartem showing mild degeneration of glomeruli.

Plate B3: Photomicrograph of fetal kidney of Group 3 whose mother received 16mg/kg of coartem showing atrophic and severely degenerated glomeruli

Plate B4: Photomicrograph of fetal kidney of Group 4 whose mother received 24mg/kg of coartem showing atrophic and severely degenerated glomeruli and acute tubular necrosis with moderate interstitial haemorrhagic hazes.

DISCUSSION

Fetal growth can be evaluated in terms of fetal weight and the crown rump length of the fetus. In this study, the fetal weight and crown rump length (CRL) of the treated groups we significantly decreased when compared to the control ($p < 0.05$). These parameters reveal fetal growth to be inversely proportional to the increasing doses of artemether/lumefantrine (Coartem) administered to the pregnant rats. The results suggest fetal growth retardation associated with administration of coartem at increasing doses. This corroborates studies where artesunate was given to pregnant Wistar rats in order to study its effect on the morphormetry of fetal central nervous system of the fetuses[24]. Fetal weight had earlier been reported to be significantly reduced ($p < 0.05$) when compared to the control following treatment of pregnant rats with some artemisinins[25]. The same study revealed a significant decrease in the sizes of the fetuses when compared to the control. Fetal weight and crown rump length have also been found to be significantly reduced following administration of aloe vera and pyrimethamine[26,27].

The liver is responsible for the metabolism of substances including xenobiotics in the system hence they become susceptible to the substances or their toxic metabolites. Drugs have been reported to induce liver toxicity and hepatotoxicity of drugs is one of the key reasons for discontinuation and removal of approved drugs from circulation[28]. Liver injury may be acute or developed over weeks to months and it may take the form of hepatic necrosis, hepatitis, cholestasis, fibrosis or liver dysfunction[1]. The present study reveals derangement and degenrations in the fetal livers of groups treated with increasing doses of artemether/lumefantrine (Coartem) from Groups 2 – 4 compared to the control (Group 1). The cellular damage was observed to be dose dependent and there was progressive cytoarchitectural damage of the fetal liver among the treated groups. Congested and dilated sinusoids and central veins, hepatic necrosis and microvesicular steatosis with vacuolations were observed in the fetal liver in this study. Phytochemicals from plants such as ginkgo biloba have been reported to be equally harmful to developing fetal liver[29]. In animal models, liver injury has been shown to result from either reactive metabolites or auto-immune mechanism in response to incoming xenobiotics[30].

Drug induced liver injury (DILI) is generally classified as direct toxicity and indirect toxicity. Direct toxicity includes injuries caused directly by the xenobiotic or its metabolites as is the case with acetaminophen[31]. Indirect toxicity is a more complicated and less understood process. It involves inflammatory process, including activation of innate and/or adaptive immune response[32]. Minor hepatocellular dysfunction and cell death caused by therapeutic drugs or other factors may trigger the activation of cells involved in the innate immune system such as kupffer cells (resident macrophages of the liver) and natural killer cells. These cells may then exacerbate an initial minor injury by activating the adaptative immune system producing pro-inflammatory cells to the liver[32].

The kidney serves as eliminatory organ for most of the drugs and xenobiotics. It removes most toxic substance from circulation and is very susceptible to toxic damage resulting from these substances. Mild to severe alterations in the architecture of the fetal kidey of rats treated with increasing doses of coartem was observed in this study. Marked distortion of fetal kidney histology has also been reported of palmwine and aloe vera[26,33]. Denegration of the glomeruli and tubular necrosis and altered liver histology in this study shows that artemether and lumefantrine can cross the placenta and affect the organs in fetuses of albino wistar rats.

Amniotic alpha fetoprotein has been used in the diagnosis of neural tube defects. In this study, the level of alpha fetoprotein is significantly reduced ($P < 0.05$) in Group 2 and Group 3 when compared to the control. The AFP of Group 3 showed significant increase when compared to the control. Elevated levels of amniotic alpha fetoprotein have been used as an indicator for fetal defect[34]. However, it is more accurate to use maternal serum and amniotic fluid samples in pregnancy to evaluate the risk of neural tube defect. This study has established that administration of artemether/lumefantrine (coartem) to pregnant albino wistar rats in second trimester can cause fetal liver and kidney toxicity as well as reduced fetal growth and body weight.

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