

HISTOPATHOLOGICAL EFFECTS OF DIFFERENT ARTEMISININ DERIVATIVES ON LIVER OF MALE ALBINO MICE

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ABSTRACT

Malaria is a common disease of humans caused by different species of plasmodium. Artemisinin derivatives like artemether and coartem are being used to treat malaria. The objective of the current study was to investigate the toxic and histopathological effects of artemisinin derivatives on the liver of male albino mice. To this end, 68 mice were randomly allocated into seven groups denoted as A-G. Group A with 8 mice served as the control, while experimental groups B, C and D each with 10 mice were given artemether intramuscularly in three doses (0.4mg/kg b.w., 0.8mg/kg b.w., 1.2mg/kg b.w.) and groups E, F and G of 10 mice each were administered coartem orally at three doses, (0.9mg/kg b.w., 1.8 mg/kg b.w., 2.7 mg/kg b.w.) for two weeks. Our findings revealed that there was a substantial decrease in the weight of the body and liver with treatment with artemether and coartem. Both drugs induced structural alterations, necrosis, deposition of fats and widening of central canal in liver. A rise in the levels of AST and ALT showed the toxic effects of artemether and coartem on the liver. We conclude that the prolonged use of artemether and coartem, even in low doses, is toxic to the body and liver of mice.

Key words: artemisinin, artemether and coartem, toxic effect, histological changes, ALT, AST.

INTRODUCTION

Malaria is a vector-borne infectious disease of humans and is mainly caused by the plasmodium species (Sarita *et al.*, 2012). Despite various control programmes, Malaria appears as one of the fatal diseases in different regions of the world (Okafor *et al.*, 2013). The recent World Health Organization statistics indicate that 214 million malaria cases and 438,000 deaths have been documented worldwide in 2015 (WHO-fact sheet, 2015). Despite an improvement in malaria control, it is still a serious problem in South East Asia, including Pakistan, neighboring countries and Africa. The issue is becoming grave due to consistent development of resistance in *Plasmodium falciparum* strains against antimalarial drugs (Leang *et al.*, 2015 and Thanh *et al.*, 2017).

Diverse therapeutic approaches have been in practice to control malaria in various parts of the world which mainly focus on the elimination of the vector of *Plasmodium falciparum* and the development of antimalarial drugs with great effectiveness against the parasite. But the development of resistance in the vector and the parasite has necessitated the formulation and discovery of new drugs. In this regard Artemisinin Combination Therapy (ACT) has been adopted worldwide as treatment of choice for uncomplicated malaria (WHO, 2015).

Artemisinin, a common antimalarial drug, is derived from the Chinese herb *Artemisia annua*. Its derivatives such as artesunate, arthemether, coartem,

sulfadoxine+pyrimethamine and mefloquine are mostly safe but can cause lethal side effects (Olliaro and Taylor, 2004; Nosten and White, 2007). Artemether has intensive antimalarial potential and is effectively exploited against drug resistant malaria (Adekunle *et al.*, 2009). Artemether is exceptionally helpful in the treatment of complicated *P. falciparum* malaria and is used widely in managing chloroquin-resistant parasites of *P. falciparum* (Murphy *et al.*, 1997; Falade *et al.*, 2005).

All drugs employed for malaria treatment have some hostile impacts on patients, with different degrees of damage (Croft *et al.*, 2002). Artemisinins in high doses can be toxic (Udobre *et al.*, 2009) and artemisinin combination therapies can affect the renal function in animals (Etim *et al.*, 2012). Similarly, the hepatotoxic effect of artesunate in guinea pigs, rabbits and rodents has been reported (Ngokere *et al.*, 2004; Nwanjo and Oze, 2007; Izunya *et al.*, 2010). The toxic effect of artesunate is not restricted to the liver and kidney but it affects the brain stem (Genovese *et al.*, 2000; Nontprasert *et al.*, 2002), stomach (Eweka and Adjene, 2008), testis and liver (Izunya *et al.*, 2010). Lumefantrine and artemether are believed to cause damage to the liver of the host and parasite both by disturbing the polymerization and synthesis of new reactive metabolites in the liver (Anyasor *et al.*, 2009; Mwesigwa *et al.*, 2010). Artemether's unfavorable impacts on other body organs have been reported in animal models (Adekunle *et al.*, 2009). Organ weight is an important criterion for evaluation of toxicological impacts. The toxic effects of

antimalarial drugs are depicted by the total and relative weight of an organ after drug administration (Maina *et al.*, 2008). It is reported that artesunate, when administered orally in Wistar rats cause lethal impact in liver cells and portal tracts (Izunya *et al.*, 2010). Similarly, malarial patients when treated with artemether, show anemia (Osonuga *et al.*, 2012). The liver is the most affected organ by administration of artemether or coartem and contains the highest quantity of ALT (Modaresi *et al.*, 2011).

Liver transaminases (AST/ALT) are biomarkers of liver function and have been investigated in humans and animal models (Lee *et al.*, 2012). AST and ALT are present in small concentrations in blood serum and their level escalates in the blood on damage to the hepatocytes (Nyblom *et al.*, 2004; Aragon *et al.*, 2010). Liver damage is caused by high concentration of ALT/AST in the serum which may be caused by different antimalarial drugs like, quinine and chloroquine etc., (Pari, and Amali, 2005; Farombi *et al.*, 2000; Debra and Megan, 1999). Artemether-lumefantrine (coartem) produces degeneration in the liver cells as evidenced by liver sections and upsurge in the level of transaminases (Solomon *et al.*, 2013; Theophilus *et al.*, 2012). Likewise, it has been shown that artemisinins, artemether-lumefantrine and halofantrine abnormally increase production of liver enzymes in animal models (Obi *et al.*, 2004; Adaramoye *et al.*, 2008).

The present study was carried out to determine the histopathological effects of two artemisinin derivatives, artemether and coartem, on male albino mice. The study evaluated the impact of these antimalarial drugs on body and organ weights, liver histology and liver function.

MATERIALS AND METHODS

Animals: Institutional Ethical Review Committee of Department of Zoology, Lahore College for Women University endorsed the study plan. Completely Randomized Design (CRD) was implemented to assess the impact of artemisinin administration on male mice.

Male albino mice were kept in wired cages for two weeks at the animal house for acclimatization. Tap water and standard feed was given to the animals. A 12hrs light/12-hrs darks cycle was maintained with an ambient temperature of 30-35°C and 65% humidity was provided. Market available mice feed was provided in the form of pellets and distilled water. Male mice (68) with average weight of 25 to 30 grams were distributed into 7 groups. Group A consisted of 8 mice and served as the control whereas Groups B to G were designated as experimental groups having 10 mice each.

Drug preparation: Two antimalarial drugs, artemether (injection) and coartem (tablets) were administered to the

experimental groups. The standard dose of artemether is 3.2mg/kg body weight and maintenance dose is 1.6mg/kg of body weight in humans (WHO 2013). In reference to this standard dose, the dose rates for experimental mice were calculated. Artemether injections were given intramuscularly to groups B, C and D in three different doses, 0.4mg/kg, 0.8mg/kg and 1.6mg/kg body weight once per diem. Coartem tablets (artemether-lumefantrine 40/240mg) (fine powder), dissolved in distilled water, were orally administered twice a day to three groups of mice (groups E, F and G) in three doses, 0.9mg/kg, 1.8mg/kg and 2.7mg/kg body weight. After 7 days of treatment, 50% mice were killed to collect blood samples and organs. At the 14th day of drug treatment, the remainders of the mice were sacrificed. The weight of the animals and the liver were recorded before and after drug treatment.

Microtomy and staining of the tissues: Formalin-fixed tissues were washed with water and dehydrated with ethanol solution in ascending order. The tissues were placed in xylene and then in pure paraffin wax. Later they were put in oven to evaporate xylene at 57°- 59° C for 12h. Subsequently, they were transferred to cavity blocks greased with egg albumin and molten wax. The blocks were cut in rectangular form, mounted and fixed on to the microtome for section cutting. The fine tissue sections were stained with hematoxylin and eosin. Part of the tissue (nucleus) stained with hematoxylin and displayed a blue color and the cytoplasm appeared pink due to eosin stain, under the microscope.

Assay procedure: To assess the impact of artemether and coartem on the liver function, two marker enzymes, AST and ALT were evaluated according to IFCC (International Federation of Clinical Chemistry) methods using ALT and AST kits by Crescent Diagnostics, Jeddah Industrial City, Saudi Arabia.

Statistical Analysis: The comparisons among the treated groups and the control group were carried out by one-way ANOVA in SPSS. Two group means (control and treated) ($P < 0.05$) were compared by "Student Newman Keuls" (SNK) test assuming following statistical model:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Where,

Y_{ij} = Observation of dependent variable on i^{th} treatment

μ = Population mean

τ_i = Effect of i^{th} treatment ($i = 1, 2, 3; \text{Control}$)

ϵ_{ij} = Residual effect of j^{th} observation on i^{th} treatment

$NID \sim 0, \sigma^2$

RESULTS

This study was done on male albino mice to investigate the histopathological effects of artemether and coartem, common anti-malarial drugs in Pakistan and

neighboring countries. The body weights of control and experimental groups were noted after 7 and 14 days of artemether and coartem treatment. Similarly, the weight of the liver of control mice was compared with liver weight of treated mice after the 1st and 2nd week of artemether and coartem administration as shown Table 1.1). The results showed that there was a consistent decrease of body weight of animals after the 1st and 2nd week of artemether administration but the decrease was less pronounced in low dose cases (0.4mg/kg & 0.8mg/kg body weight) than high dose cases (1.2mg/kg). However, the effect of coartem on body weight was significant when compared with the control but less pronounced when compared with artemether administration (Fig. 1A, B).

The effect of artemether and coartem on liver weight was also recorded. The results showed that liver weight underwent a consistent decrease after one and two weeks of both drug treatments. Both drugs induced almost similar amounts of weight decrease in the liver (Fig. 1C, D).

Histopathological Studies

Histology of control liver: The liver micrograph of control group showed the normal histological features of hepatocytes (h) with clear cytoplasm and normal central nuclei. There was no vacuole formation in hepatocytes and the central vein (cv) was normal without any damage. The liver was working properly as no fat deposition was observed (fig. 2 A).

The results described below for artemether/coartem administration were based on the observations made in majority of the animals. In each experimental group there were 10 mice, half (five) were sacrificed after seven days while the remainder were killed after 14 days of treatment. The effect caused by artemether/ coartem was recorded only if it was found in minimum of three out of five mice in each group.

Histology of liver after 7 days of artemether treatment: Histopathological changes after one week of administration of different concentrations of artemether

were studied in all groups. Group B (0.4mg/kg) showed normal hepatocytes with slight changes in nuclei. Group C (0.8mg/kg) exhibited irregular sized hepatocytes indicating the start of degeneration. The group D (1.2mg/kg) showed loss of specific arrangement of hepatocytes mainly around the central channel with fat deposition (Fig. 2B, C, D).

Histological changes in liver after seven days of coartem treatment: The liver micrograph of group E with low dose (0.9mg/kg) showed slight changes in the liver and cell structure. However, in group F (1.8mg/kg) hepatocyte organization was affected and fewer cells showed necrosis (N). Group G (2.7mg/kg) showed changes in hepatocyte arrangement with sinusoidal enlargement and necrosis. The comparison of the two drugs showed that the damage to the liver under coartem treatment was less pronounced as compared to artemether administration (Fig. 2E, F, G).

Histological changes in liver after 14 days of artemether treatment: The micrograph showed visible lesions in the group B administered with artemether (0.4mg/kg) for two weeks. The hepatocytes were clearly of irregular shape. They showed recognizable banded hepatic cord. The group C (0.8mg/kg) revealed more intensive alterations like widening and enlargement of the sinusoidal linings due to damaged parenchyma. Group D (1.2mg/kg) showed visible necrosis in the hepatocytes along with a damaged central vein (Fig. 3B.C.D).

Histological changes in liver after 14 days of coartem treatment: The results in the coartem-treated groups showed less pronounced changes as compared to the changes reflected by artemether-treated groups. Group E (0.9mg/kg) showed visible necrosis of the hepatocytes with disintegration of nuclei. The group F (1.8mg/kg) revealed the loss of liver architecture and the sinusoidal congestion. In group G (2.7mg/kg) the hepatocytes showed fat deposition and most of the cells faced intensive necrosis. The central vein in coartem-treated animals presented noticeable damage (Fig. 3E.F.G).

Table 1. Effect of artemether and coartem on Body and Liver weights after 7 and 14 days of treatments

Groups	After 7 Days of treatment		After 14 days of treatment	
	Body weight (gm)	Liver weight (gm)	Body weight (gm)	Liver weight (gm)
Control	29.68±0.4	5.05±0.048	29.68±0.4	5.05±0.048
Artemether 0.4 mg/kg	27.35±0.39	4.96±0.054	26.83±0.57	4.80±0.066
Artemether 0.8mg/kg	26.75±0.65	4.85±0.054	*25.96±0.52	4.64±0.057
Artemether 1.2mg/kg	*25.31±0.48	*4.70±0.050	*24.76±0.51	*4.50±0.067
Coartem 0.9mg/kg	28.80±0.34	4.96±0.051	27.28±0.41	4.81±0.033
Coartem 1.8mg/kg	28.1±0.33	4.83±0.056	26.69 ±0.50	4.71±0.025
Coartem 2.7mg/kg	27.82±0.36	*4.75±0.051	*26.20±0.51	*4.60±0.035

Values are given as means ± SEM for 10 rats in each group. * significantly different P<0.05 compared to control (n=8)

Liver function tests: To assay the liver function, ALT and AST were determined before and after treatment with artemether and coartem. The results revealed that there was a consistent increase in AST and ALT levels after one and two weeks of artemether and coartem treatment and this increase was dose and time dependent as

compared to the control group. The increase in ALT and AST level was less pronounced in artemether groups, particularly after two weeks of drug treatment, than the levels of ALT/AST in the coartem-treated groups (Table 2).

Table 2. Effect on serum AST and ALT levels after 7 and 14 days of artemether and coartem administration

Groups	Liver function tests after 7 Days of treatment		Liver function tests after 14 days of treatment	
	ALT U/L	AST U/L	ALT U/L	AST U/L
Control	0.0050±0.0003	14.36±0.30	0.0050±0.0003	14.36±0.30
Artemether 0.4 mg/kg	0.0058±0.00028	15.08±0.33	0.0068±0.00028	16.93±0.15
Artemether 0.8 mg/kg	0.0063±0.00030	15.67±0.18	0.0077±0.00038	*17.57±0.16
Artemether 1.2 mg/kg	*0.0072±0.00028	*16.64±0.15	*0.0088±0.00028	*18.26±0.08
Coartem 0.9 mg/kg	0.0057±0.00030	14.97±0.36	0.0068±0.00028	16.80±0.15
Coartem 1.8 mg/kg	0.0058±0.00028	15.57±0.20	0.0075±0.0002	17.35±0.95
Coartem 2.7 mg/kg	0.0065±0.00020	16.49±0.16	*0.0080±0.00024	*18.01±0.10

Values are given as means ± SEM for 10 rats in each group. * significantly different P<0.05 compared to control (n=8)

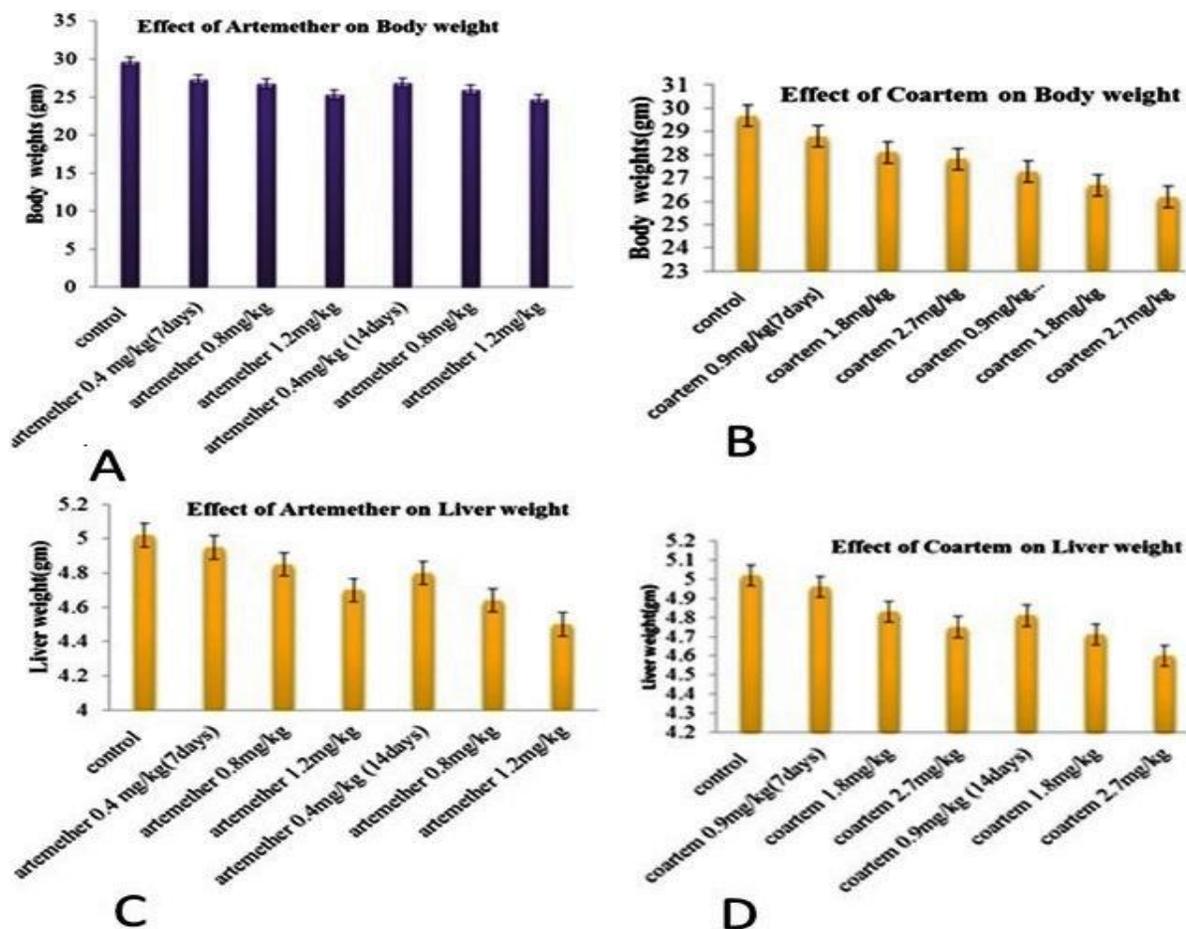


Figure 1: Effect of Artemether and Coartem on liver and body weights of mice:

Group B-G were treated with different doses of artemether (0.4mg/kg b.w., 0.8mg/kg b.w., 1.2mg/kg b.w.) and coartem (0.9mg/kg b.w., 1.8 mg/kg b.w., 2.7 mg/kg b.w.) for 7 and 14 days as shown. The body and liver weights were measured and recorded after stipulated time period. A is from the untreated control group.

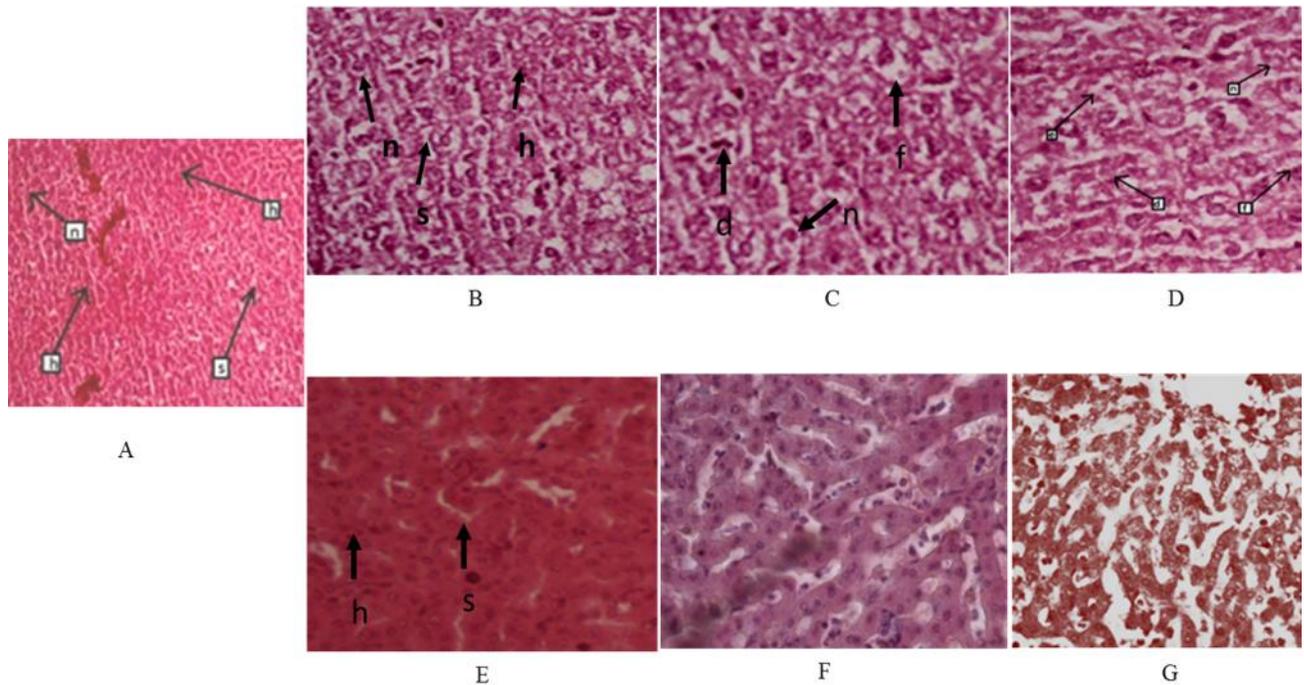


Figure 2: Effect of Artemether and Coartem on mice liver histology after 7days of drugs treatment:

Group B-G were treated with different doses of artemether (0.4mg/kg b.w., 0.8mg/kg b.w., 1.2mg/kg b.w.) and coartem (0.9mg/kg b.w., 1.8 mg/kg b.w., 2.7 mg/kg b.w.) for 7 days as shown. The liver sections showed hepatocytes, sinusoids and central canal with pronounced change; (h) hepatocytes, (s) sinusoid, (n) nucleus, (f) fat deposition (Mag. X400).

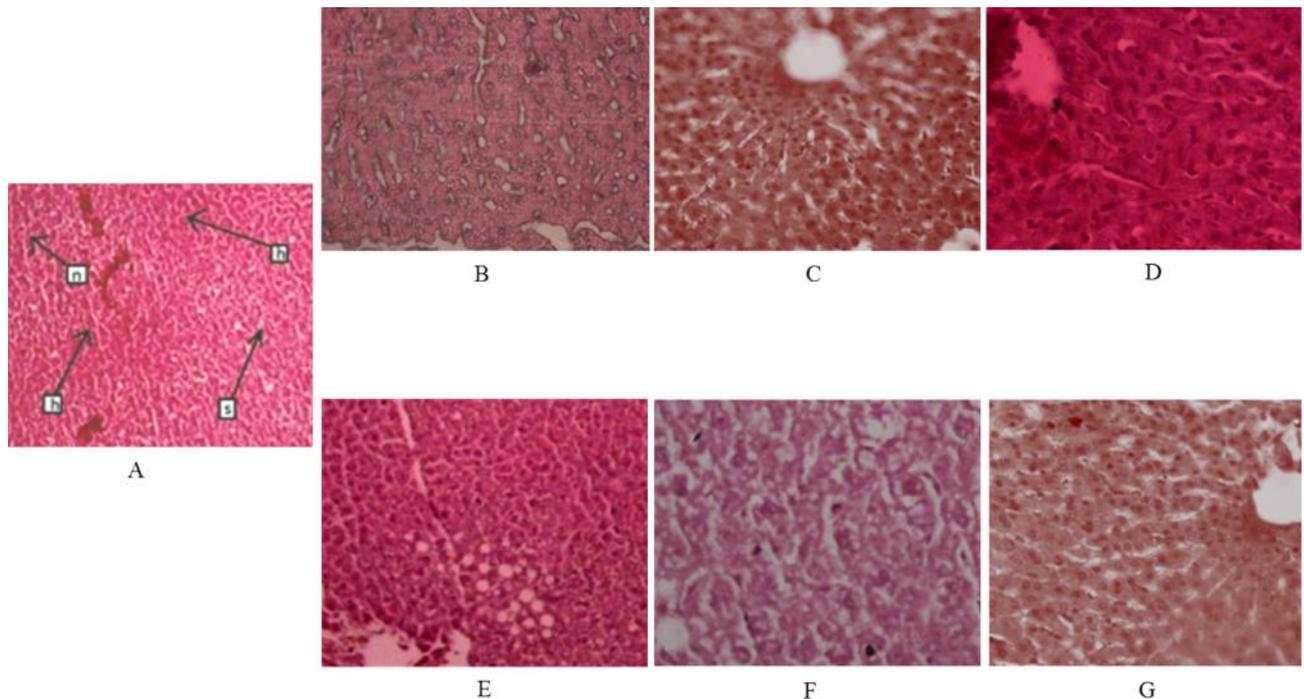


Figure 3: Effect of Artemether and Coartem on mice liver histology after 14days of drugs treatment:

Group B-G were treated with different doses of artemether(0.4mg/kg b.w., 0.8mg/kg b.w., 1.2mg/kg b.w.) and coartem (0.9mg/kg b.w., 1.8 mg/kg b.w., 2.7 mg/kg b.w.) for 14 days as shown. The liver sections showed hepatocytes, sinusoidsand central canal with pronounced changes like necrosis, fat deposition and alteration in central vein; (h) hepatocytes, (s) sinusoid, (n) nucleus, (f) fat deposition (Mag. X400).

DISCUSSION

Malaria is a common and dangerous disease of Africa and Asia. Multiple antimalarial drugs are in use to curb the dissemination of malaria. These antimalarial drugs cause some harmful effects generally and even entail critical impact on body and organs (Croft *et al.*, 2002). In the present study we examined the effects of artemether and coartem, two antimalarial drugs, administered to the male albino mice. Artemether and coartem are widely used against malaria all over the world.

The current study evaluated the lethal impact of two antimalarial drugs, artemether and coartem, on the body weight and liver of mice. There was a reduction in the body weights of animals under the administration of both artemether and coartem. The decrease was more pronounced after 14 days of drug treatment and organ weights also decreased. Our findings are in agreement with the findings of Rajiet *et al.*, (2005) who reported a decrease in the body weight of animals administered with artemether. Moreover, Rajput *et al.* (2012) showed similar results in mice when another artemisinin derivative, artesunate, was administered. Our results are further supported by many investigators who reported a significant decrease in the body and organ weights in response to antimalarial drugs such as chloroquine (Okanlavon and Ashiru, 1998; Zahid and Abidi, 2003). Our findings question the results of Solomom, *et al.* (2013) who reported that oral administration of coartem did not change the body weights of rats significantly.

The present study showed damage to the hepatocytes under treatment with artemether and coartem. There was enlargement of the sinusoidal lining with noticeable damage of the central vein. Our findings are consistent with the report of Solomon *et al.* (2013) who revealed the lethal effect of coartem on hepatocytes of male Wistar rats. Hepatocyte damage in response to the treatment of artemether and coartem was also demonstrated by Rajput *et al.* (2012) who showed similar results with the administration of artesunate. Similar results were reported by Negokere *et al.* (2004) who documented the increase in the hepatic enzymes of rabbits with administration of artesunate.

The liver function tests were performed to examine the damage caused by artemether and coartem. Our findings revealed that artemether and coartem treatment induced significant increases of ALT and AST. Our results are in agreement with the work of Okunlola *et al.* (2013) who documented increases in AST and ALT levels after administration of artemisinin derivatives and hepatic damage in mice. Our findings are supported by the work of other researchers (Farombi *et al.*, 2000; Obi *et al.*, 2004; Nyblom *et al.*, 2004; Pari and Anuli, 2005) who showed an increase of serum enzymes in response to antimalarial drugs.

Our results are in contrast to the findings of Adaramoye *et al.* (2008) who showed no significant increase in AST and ALT levels with the treatment with artemether-lumefantrine (coartem). However, our findings are further supported by the work of many investigators who reported hepatic damage due to increase in serum enzymes by administration of halofantrine (Obi *et al.*, 2004), amodiaquine (Farombi, 2000), sulfadoxin pyrimethamine (Mishra *et al.*, 2011) and chloroquine (Pari and Amali, 2005). Similarly, hepatotoxicity in response to artemether-lumefantrine and escalation in AST and ALT level was reported elsewhere (Solomom *et al.*, 2013). Our findings are also consistent with results of Theophilus *et al.* (2012) who documented the higher levels of AST, ALT and ALP in response to treatment with coartem.

Conclusion: We conclude that artemisinin derivatives like artemether and coartem, when administered to mice in low and high doses for one and two weeks, cause side effects in terms of loss of organ and body weights. Both drugs induce structural alterations and damage to the liver. The increase in AST and ALT shows that the liver undergoes damage due to consistent use of the artemisinin derivatives. It is seen that artemether and coartem are not well tolerated by mice even at certain low doses.

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