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RESEARCH ARTICLE
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STUDY ON EFFECTS OF ARTEMETHER-LUMEFANTRINE AND IBUPROFEN ON LIVER OF RABBITS

Farheen Bhatti[1], Safdar Ali Ujjan[1], Sajid Ali[2*], Sham Lal[3], Wali Muhammad Mangrio[1], Zaibun-Nisa Memon[1], Kiran Naz sahito[1]

[1]Department of Zoology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
farheenbhatti14@gmail.com, safdar.ujjan@salu.edu.pk, wali.mangrio@salu.edu.pk, zaib.nisa@salu.edu.pk, nazkiran392@gmail.com
[2*]Department of Pharmacy, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
sajid.mojai@salu.edu.pk
[3]Department of Microbiology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan,
shamlal@salu.edu.pk

[*]**Corresponding Author:** Sajid Ali
[*]Department of Pharmacy, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
sajid.mojai@salu.edu.pk

Abstract:
Artemisinin based combination therapy (ACT) is reposed to be employed by WHO. This therapy consists of a derivative of artemisinin and associated drugs. Artemether lumefantrine administration though produces free radicals that eventually cause damage to the cells with evidence of both toxicity of liver, heart, kidneys and other organ toxicity. In this study 12 rabbits were included and were assorted into four groups A, B, C and D. Each group contained two animals. Group A was control group and maintained at normal diet and water. Group B, C and D were experimental groups and given artemether-lumefantrine ibuprofen and combined art-lum and ibuprofen respectively. At the end of experiment and before sacrifice animals were fasted overnight. Blood was collected from the aorta through cardiac puncture into EDTA containing tubes. The clear plasma was obtained and used for enzyme analysis. The biochemical assay included glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) to tests the state of liver. The microtome machine was used to cut and section the liver tissue at size of 6-8µm thick. The slides were prepared and stained with dyes such as hematoxylin and eosin. For histopathological studies, the tissue slides were observed under microscope and photos of tissue sections were taken using camera fitted microscope in the department of Zoology, SALU. The administration of both drugs, artemether-lumefantrine and ibuprofen individually and combined showed histopathological, biochemical and clinical effects on animals. The elevation of serum ALT, AST and ALP enzymes is found in animals treated with both drugs individually and combined. The increased in eosinophil mass and inflammation has been observed in individual drug administration while severe lymphocytic infiltrates and triditis has been found in combined drug administration. General decrease in weight of animal was also observed after the course of drug administration.

Keywords (in English): Artemether, Lumefantrine, Ibuprofen, Artemisinin.

1 INTRODUCTION

1.1 Artemisinin Based Combination Therapy (ACT)

Artemisinin is isolated from *Artemisia annua*, a medicinal plant present in china, and is used as natural anti-malarial drug (Hatz *et al.*, 2008). The genre Artemisia L. includes in Composite family. Above 350 species of Artemisia are discovered, which are used in customary kindred medications for numerous uses such as various allergies, blood circulation, cough and cold, polyuria, high blood pressure, and parasites, etc (Tan *et al.*, 1998). The effective component artemisinin synthesized in 1972 from *Artemisia annua* as well as other semi-synthetic medicines were formed from it (Bigoniya *et al.*, 2015). These additional prepared products of artemisinin comprise: artemether (artemotil), artemether, artesunate and artenimol (â-dihydroartemisinin, DHA) (Pukrittayakamee *et al.*, 2004). ACT is reposed to be employed by WHO. This therapy consists of a derivative of artemisinin and an associated drug; the decay rate of artemisinin component is comparatively short and acts rapidly to decrease parasite load, on the other hand associated drug has long decay rates that burke parasitaemia up to weeks after treatment (Davlantes *et al.*, 2018).

1.2Artemether-lumefantrine combination

About 20mg artemether and 120mg lumefantrine as a standard tablets or dispersible tablets are used as a fix dose combination. Recommended dosage of artemether-lumefantrine according to WHO is 6 tablets dose administration two times daily for 3 days (1^{st} two dosages should be administered after 8-hour time interval) with target dose range of 5-24 mg of artemether per kilogram of weight and 29-144mg of lumefantrine per kilogram of weight (WHO, 2015). A/L (Artemether lumefantrine) administration, though produces free radicals that eventually cause damage to the cells with evidence of both toxicity of heart, toxicity of kidneys and other organ toxicity (Efferth *et al.*, 2010).

1.3 Pharmacology of ibuprofen

Ibuprofen is (2RS)-1[4-(2-methyl propyl) phenyl] propionic acid (Bradbury, 2004). It was presented in 1916 and is the first among propionic acid products which is verified asmore effective substituteof Aspirin (Tripathi, 2003). It is a common nonsteroidal anti inflammatory drug (NSAID). It gives relief in the pyrexia and common ailment of cold and is deliberated as one of the harmless NSAIDs (Hendley, 2011). There are also some side effects of ibuprofen and the very common side effects are, general discomfort of stomach, nauseousness and vomit, while not so much as aspirin or indomethacin (Tripathi, 2003). At first, the medicine was used in lower dosagesextended from 400 to 1,200 mg/day with the range and knowledge by doctors' careful dose rise as now it is advanced to the today's suggested dose of 2,400 mg per day. The most important concern on careful administration of ibuprofen was a symbol of its initial victoryand raising account that it was innocuous (Rainsford, 1999).

2 Materials and Methods

2.1 Animals and animal care

12 Rabbits were purchased from Sukkur market. The animals were kept in well ventilated metallic/wooden cage at normal room temperature about $28\text{-}36^0$C and 12 hours light/dark cycle. They were maintained at standard diet and tap water *ad libitum* (Bigoniya *et al.*, 2015).

2.2 Drugs and reagents

Artemether-lumefantrine tablets (coartem) and ibuprofen tablets was purchased fromsukkur and reagents were included: paraffin wax, Ethanol, xylene, eosin dyehaematoxylin dye, phenol, phosphate buffer (p^H 7.4), EDTA, Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphate ALP) assay kits (Omotuyi *et al.*, 2008). All chemicals were of analytical grade.

2.3 Drug administration

The animals were assigned into four groups A, B, C, and D, each group contained three animals. Group A was control group having standard diet and tap water *ad libitum*. All the other three groups

B, C, D were experimental groups. Group B was given art-lum (coartem) at therapeutic dose (14mg/kg body weight) dissolved in distilled water twice a day for 3 days (Francis *et al*., 2008). The group C received ibuprofen at curative dosage of 40mg/kg body weight for 3 days and the fourth group D was given art-lum 14mg/kg bw and 40mg/kg bw of ibuprofen for 3 days.

2.4 Clinical Assessment

After the course of study, the clinical signs were determined such as weight loss or any general weakness and hair loss was observed. At the start of experimentation weight of each animal in all four groups was measured and after the respected drug administration period, weight was measured so as to observe any decrease in weight of animal.

2.5 Sample preparation

Animals were sacrificed fasted over night after receiving therapeutic doses of respected drug. Euthanisation was done after ether anesthesia. Blood was collected from cardiac puncture from the aorta into EDTA containing tubes (Asiedu*et al.*, 2016). Blood was centrifuge at 3000×g for 15 minutes and clear Serum was separated. The clear plasma obtained was used for enzyme analysis (Adaramoye*et al.*, 2008).

2.6 Biochemical assay

Biochemical assay of the serum prepared and stored in EDTA was analyzed. The Biochemical assay included, glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). These liver function tests were indicating the state of liver (Asiedu*et al.*, 2016). Biochemical assay will be performed in the laboratory at Shah Abdul latif University Khairpur.

2.7 Histopathology

Rabbits were sacrificed and organs (livers) were removed swiftly and bathed with 0.9% saline. The livers were kept in solution of 10% neutral formalin buffer. The microtone machine was used to cut and sectioned the liver and each slice was of 4-5µm thick. The slides were prepared of tissue slices and stained with dyes such as haematoxylin and eosin. For pathological studies, the tissue slides were observed under microscope and photos of tissue slides were taken using camera (Bhandari*et al.*, 2017, Francis *et al.*, 2013, Asiedu *et al.*, 2016).

2.8 Statistical Analysis

Data wasset in table using Microsoft Excel and transferred to SPSS version 21 for analysis through *t* test to evaluate the results. Differences at $p<0.05$ was considered as significant.

3 Results

Present study was conducted on rabbits as experimental animals to observe any side effects or safe use of artemether lumefantrine and ibuprofen medication on liver. The study was conducted in January 2020

3.1 Comparative levels of AST, ALT and ALP enzymes of control group and treated group (group B).

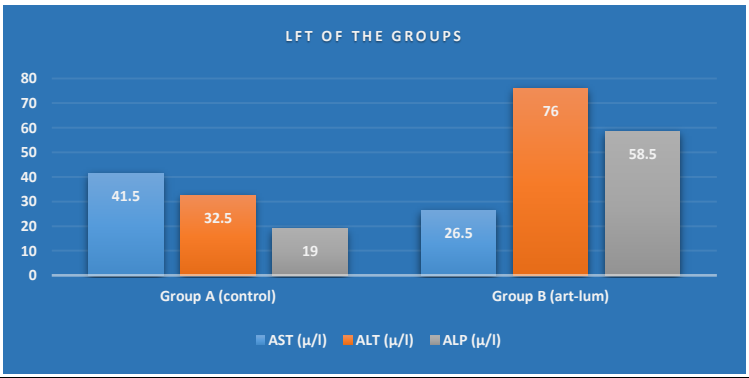


Figure 1. Showing the comparison of levels of AST, ALT and ALP in control group (untreated group) and group treated with artemether lumefantrine. The level of AST slightly decreased in experimental group while the level of ALP increased in experimental group and the level of ALT significantly increased in treated group as compared to control group. This indicated that artemether lumefantrine has adverse effects on enzymes released by liver.

3.2 Comparative levels of AST, ALT and ALP in control group and treated group (group C)

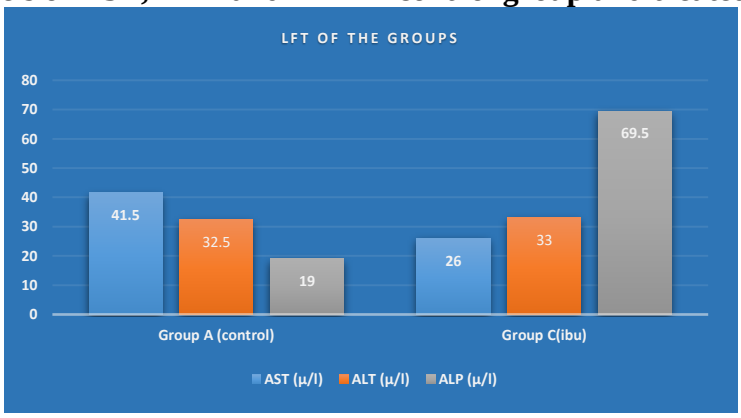


Figure 2. This graph shows comparison of levels of LFT between control and treated group with Ibuprofen. There was no significant difference in the levels of AST and ALT between control and treated groups. While the level of ALP significantly increases in ibuprofen group. This indicates that the ibuprofen has profound effect on ALP levels.

3.3 Comparative levels of AST, ALT and ALP in control group and treated group (group D)

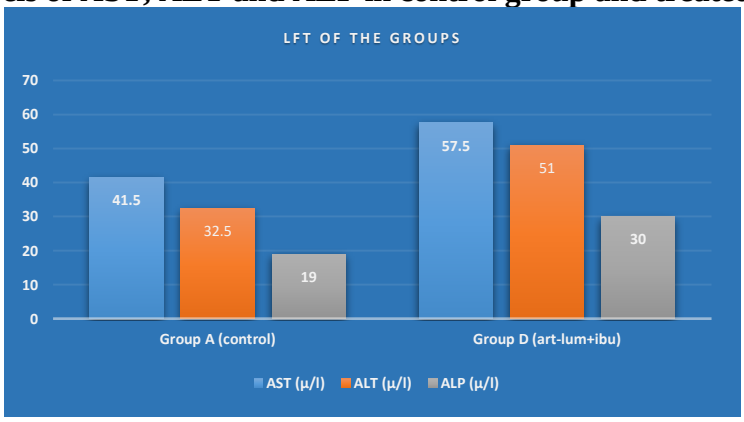


Figure 3. Shows the comparison of levels of AST, ALT and ALP in control group and experimental group (group D) treated with artemether lumefantrine and ibuprofen. The graph shows that the level of ALP slightly increases in treated group while the levels of ALT and AST markedly increase in treated groups as compared to control group. This shows that the combined drugs has effects on levels of ALT and AST enzymes.

3.4 Comparative levels of AST, ALT and ALP in control group and all treated groups (group B,C and D)

	AST (µ/l)	ALT (µ/l)	ALP (µ/l)
Group A (control)	41.5	32.5	19
Group B (art-lum)	26.5	76	58.5
Group C (Ibu)	26	33	69.5
Group D (art-lum+ibu)	57.5	51	30

Table 1. Levels of AST, ALT and ALP enzymes in all groups

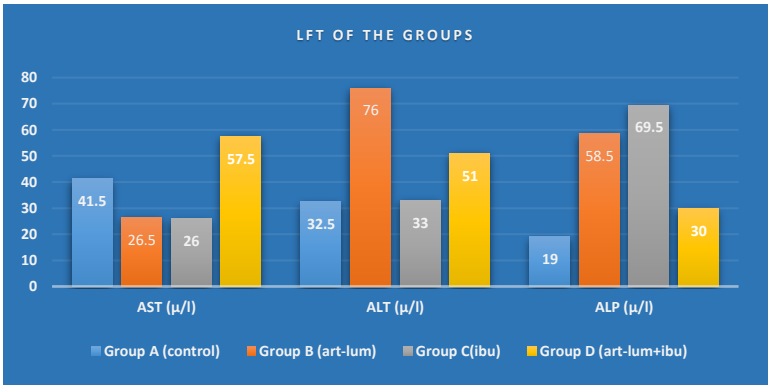
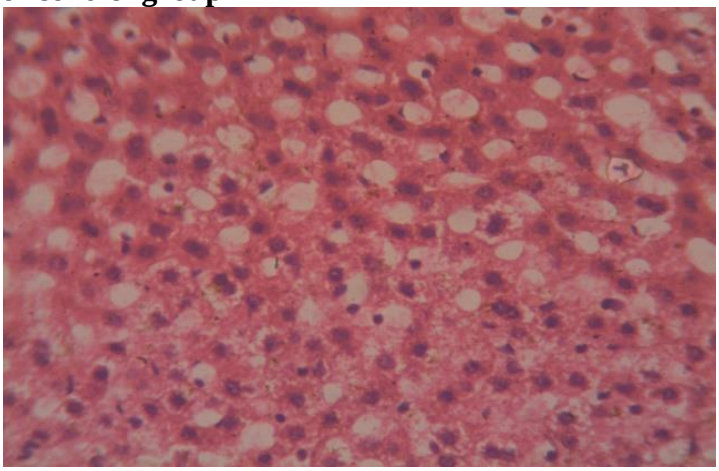


Figure 4. Shows the comparison of levels of ALT, AST and ALP in control and all treated groups. The level of ALT is highest in B group (treated with art-lum) among all groups while the level of ALP is highest in C group (treated with Ibuprofen) and the level of AST is highest in group D (treated with combination of artemether lumefantrine and ibuprofen).

3.5 PHOTOMICROGRAPHS OF LIVER

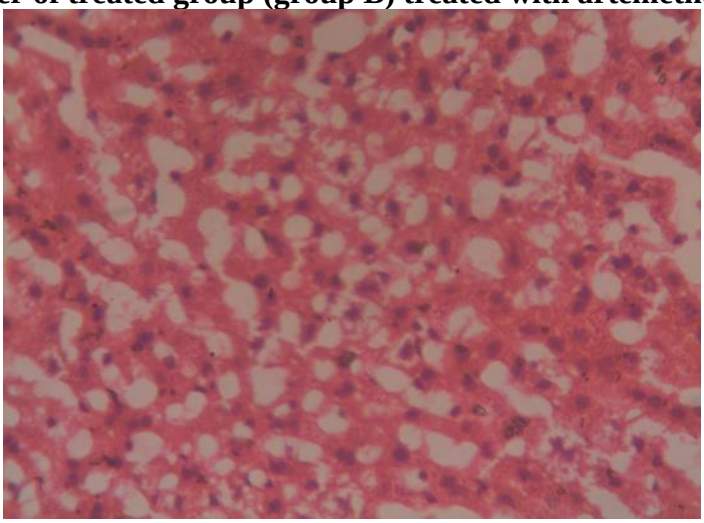
Micrograph of liver of control group



H and E 100

Figure 5. Micrograph of control group showing normal lobules and normal parenchyma.

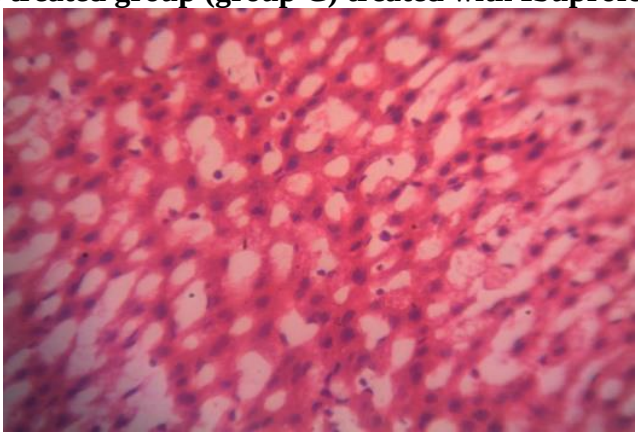
3.6 Micrograph of liver of treated group (group B) treated with artemether lumefantrine



H and E 100

Figure6. Micrograph of liver treated group, treated with art-lum at the dose of 14mg per kg body weight for 5 days shows that there is inflammation and mild increase in eosinophil mass has been observed.

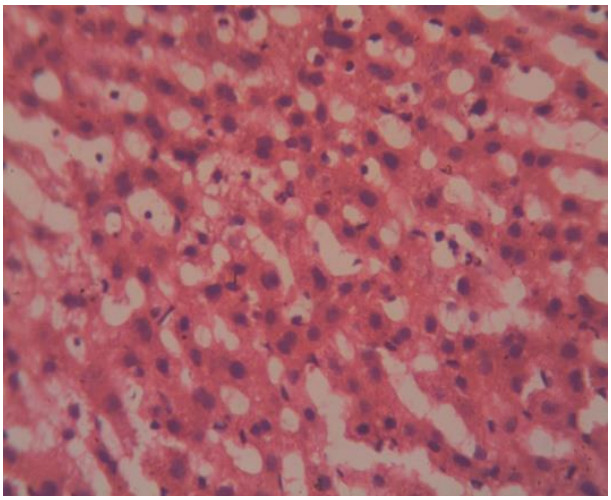
3.7 Liver micrograph of treated group (group C) treated with Ibuprofen



H and E 100

Figure 7. Liver micrographs of treated group (group C), treated at the dose of 40mg per kg body weight of Ibuprofen for 5 days, shows that there is increase lymphocytic infiltration means increase of White blood cells and inflammation has been observed.

3.8 Liver micrographs of treated group (group D) treated with combined dose of art-lum and Ibuprofen



H and E 100

Figure 8. Micrographs of treated (group D) treated with combination artemether lumefantrine and ibuprofen at the dose of 14mg/kg body weight of art-lum plus 40mg of ibuprofen shows that there is severe increase of lymphocytic infiltrates and severe triditis means inflammation has been observed.

Discussion

Artemisinin is isolated from *Artemisia annua*, the medicinal plant present in china, and is used as natural anti-malarial drug (Hatz *et al.,* 2008).Artemisia used in customary kindred medications for numerous uses such as various allergies, blood circulation, cough and cold, polyuria, high blood pressure, and parasites, etc (Tan *et al.*, 1998).

Artemether-lumefantrine combination is extensively used to treat malaria, various experiments have been performed for its safety use on animals for instance; rats, rabbits, dogs and guina pigs and their effects were studied.

As other agents, artemisinin may not be free of side effects or toxicities in both human and animal study. Toxicity in animals have also reported such as neurotoxicity (Brewer *et al*, 1994).

The effects of ALC are constant in similar studies evaluate the toxicity of DHA in rats; Utoh-Nedosa (2009) reported that the serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzymes levels of DHA administered rats had no significant effects. However, these results are at difference with other findings of artesunate and DHA, which

described that artemisinin derivatives cause rises in the serum levels of liver enzymes (Ngokere *et al*, 2004).

Artemether lumefantrine combination at the normal dose of 14mg/kg body weight for 5 days showed increase in serum levels of ALP and ALT enzymes.

Administration of Ibuprofen at the dose of 40mg/kg body weight for 5 days showed elevation of serum levels of ALP enzyme.

The combined administration of artemether lumefantrine and ibuprofen at the dose of 14mg/kg bw of art-lum and 40mg/kg bw of ibuprofen to test animals for 5 days showed elevation of serum levels of AST, ALT and ALP enzymes.

Histopathological studies showed that the artemether lumefantrine administration at the normal dose of 14mg has effects on state of liver resulted in increased mass of eosinophil and swelling in liver.

Administration of ibuprofen at normal dose of 40mg showed increase in lymphocytic infiltration and inflammation of liver.

Combined administration of artemether-lumefantrine and ibuprofen at normal doses resulted in acute lymphocytic infiltrates and triditis of liver.

The drug administration in all groups showed some clinical effects such as decrease in weight and hair loss.

5.2 Conclusion

The administration of both drugs, artemether-lumefantrine and ibuprofen individually and combined to test animals showed histopathological, biochemical and clinical effects on animals.

The elevation of serum ALT, AST and ALP enzymes is found with both drugs individually and combined.

The increased in eosinophil mass and inflammation has been observed in individual drug administration while severe lymphocytic infiltrates and triditis has been found in combined drug administration.

General decrease in weight of animal also observed after the course of drug administration.

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