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Effects of Artemether-Lumefantrine and Naproxen Combination on Liver and Kidney of Rats

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Abstract: *The effect of therapeutic doses of Artemether-Lumefantrine and Naproxen combination on kidney and liver was investigated. Twelve albino rats were randomly assigned into four groups (Control, Art-Lum, Nap and Art-Lum+Nap) of three animals each. DMSO (5%), Artemether-lumefantrine (2.29mg/13.71mg/kg body weight), Naproxen (12.50mg/kg body weight) and combination of Artemether-Lumefantrine and Naproxen (2.29mg/13.71mg/kg body weight and 12.50mg/kg body weight) were administered orally, twice daily for three days, to animals in Control, Art-Lum group, Nap group and Art-Lum+Nap groups. Enzyme activities (alanine aminotransferase {ALT}, aspartate aminotransferase {AST} and alkaline phosphatase {ALP}) and serum macromolecules levels (Urea, Creatinine, Total protein, Albumin and Globulin) were assayed for. Results indicated that the combination of the drugs brought about increase in creatinine level, activities of ALT, ASP and ALP in serum compared to the control. A significant decrease ($p<0.05$) was observed in the kidney-body weight ratio, serum total protein, ALT activity in kidney and ALP activity in kidney compared to control. There were no significant changes ($p>0.05$) in the urea level, globulin level, albumin level, ALT activity in liver, AST activity in liver and kidney and ALP activity in liver relative to the control. The combination of the drugs may lead to renal constriction and malfunction. Also hepatic function distortion may arise. The adverse effect of the drugs appeared more pronounced in the kidney than the liver. The drugs, therefore, should be used with caution especially by patients with compromised renal functions.*

Keywords: *Artemether-lumefantrine, Kidney, Liver, Malaria, Naproxen, NSAIDs*

I. Introduction

Malaria remains one of the most widespread parasitic tropical diseases [1]. An estimated 3.4 billion people were at risk of malaria in 2012 with an estimated 207 million cases of malaria worldwide [2]. Artemisinin-based combination therapies are recommended as the first-line treatment of malaria caused by *P. falciparum*, the most dangerous of the Plasmodium parasites that infect humans and artemether-lumefantrine continued to account for the largest volume of artemisinin-based combination therapy (ACT) procured by the public and private sector [2].

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent one of the most widely used classes of drugs, and are used primarily for treatment of osteoarthritis, rheumatoid arthritis and other inflammatory disorders [3]. The basic mode of action of NSAIDs is inhibition of the pro-inflammatory enzyme cyclooxygenase (COX) [4] which exist as two isozymes denoted as COX-1 and COX-2. COX-1is made constitutively in most tissues, and is required for maintenance of healthy gastric tissue, renal homeostasis, and platelet aggregation whereas COX-2 is inducible in a limited number of tissues in response to products of activated immune and inflammatory cells. NSAIDS inhibit both COX-1and COX-2 and, thus, prevent the synthesis of prostaglandin [5].

Although effective at relieving pain and inflammation, NSAIDs are associated with a significant risk of serious gastrointestinal adverse events with chronic use [6]. Naproxen is one of the most used NSAIDs for the treatment of arthritic pain [7] and it's commonly recommended on the basis of extensive evidence supporting efficacy and safety [4]. Naproxen has been reported to be the NSAID that presented the least cardiovascular risk and providing the most effective relief for arthritic patients [8-9].

For the concurrent management of chronic/persistent pain and malaria, NSAID such as naproxen and antimalarial agent such as artemether-lumefantrine are commonly prescribed. Therefore, this research was designed to evaluate the safety or otherwise of this combination with respect to the liver and kidney.

II. Materials and Methods

2.1 Drugs and Reagents

Artemether-Lumefantrine tablets (Novartis Pharmaceuticals Corporation, USA) and Naproxen Tablets (Maydon Pharmaceuticals Ltd., Nigeria) were used. Assay kits, obtained from Randox Laboratories Ltd (Antrim UK), were used. All the other reagents used for this study were of analytical grade.

2.2 Animals and Animal Care

Twelve albino rats (*Rattus norvegicus*) of average weight of 120 ± 10 g were purchased from the Animal Breeding Unit of Department of Pharmacology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The animals were housed in standard plastic cages and acclimatized for 7 days in a well-ventilated room at room temperature of 28.0 ± 2.0 ^{0}C under natural lighting condition. They were allowed standard rodent feed and water *ad libitum*. Minimal yet statistically sufficient replicates were used to avoid animal wastage.

2.3 Experimental design

After days of acclimatization, the animals were randomly assigned into four groups (Control, Art-Lum, Nap and Art-Lum+Nap) of three animals each. The drugs were homogenized and dissolved in 5% DMSO. Animals in Control group received 0.3 ml of 5% DMSO. Artemether-Lumefantrine (2.29mg/13.71mg/kg body weight) was given to animals in Art-Lum group, Naproxen (12.50mg/kg body weight) was given to animals in Nap group. The combination of Artemether-Lumefantrine (2.29mg/13.71mg/kg body weight) and Naproxen (12.50mg/kg body weight) was administered to the animals in Art-Lum+Nap group. These doses are equivalent to the therapeutic doses of the drugs. These drugs were administered, orally, as homogenous suspensions twice daily (12 hours interval) for three days.

2.4 Sample Preparation

The animals were fasted overnight and sacrificed under mild anaesthesia using diethyl ether. Jugular venous blood was collected from the experimental animals. The serum was prepared by centrifuging the clotted blood samples at 3000 rpm for 5 min within 1 h of blood collection and collected by pippeting. The animals were also quickly dissected and the organs (liver and kidney) were excised. The organs were suspended in ice-cold 0.25 M sucrose solution (1:5 w/v) and homogenized. The homogenates were kept frozen overnight to ensure maximum release of the enzymes [10]. The homogenate were centrifuged 1500 rpm for 10 min to obtain the supernatants.

2.5 Assay of biochemical profiles

Urea was determined by Urease-Berthelot method as described by Weatherburn [11]. Creatinine was determined as described by Bartels and Bohmer [12]. Total protein was determined by Biuret method as described by Gornall *et al* [13]. Albumin was determined by Bromocresol Green method as described by Doumas *et al* [14]. Globulin was determined by the subtraction of albumin from Total protein. Aspartate aminotransferase and alanine aminotransferase activities were estimated according to method described by Reitman and Frankel [15] and alkaline phosphatase activity was determined by colorimetric method according to the recommendations of [16]. Percentage organ-body weight ratio was obtained mathematically by finding the ratio of weight of excised organ to the weight of the corresponding rat.

2.6 Statistical Analysis

Data were analyzed for statistical significance by one-way analysis of variance followed by Duncan's post hoc multiple comparisons, using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Differences at $p< 0.05$ were considered significant.

III. Results

The combined administration of Artemether-Lumefantrine and Naproxen did not significantly change ($p>0.05$) the liver-body weight ratio but significantly decreased ($p<0.05$) the kidney-body weight ratio relative to the control (Table 1).

Table 1: Effects of Artemether-Lumefantrine and Naproxen combination on the percentage organ-body weight ratio of rat

	% Organ –Body Weight Ratio	
Group	**Liver**	**Kidney**
Control	3.94 ± 0.24^{a}	0.64 ± 0.01^{b}
Art-Lum	4.05 ± 0.21^{a}	0.62 ± 0.03^{ab}
Nap	4.14 ± 0.20^{a}	0.62 ± 0.01^{ab}
Art-Lum + Nap	3.97 ± 0.19^{a}	0.60 ± 0.02^{a}

Values are expressed as Mean ± S.D (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

The combination of Artemether-Lumefantrine and Naproxen significantly increased ($p<0.05$) the Creatinine level compared to the control but did not significantly change ($p>0.05$) the Urea level (Table 2).

Table 2: Effects of Artemether-Lumefantrine and Naproxen combination on Creatinine level and Urea level in rat Serum

Group	**Creatinine (µmol/l)**	**Urea (mmol/l)**
Control	53.63 ± 7.74^{a}	5.24 ± 1.33^{a}
Art-Lum	183.23 ± 19.48^{b}	5.52 ± 1.34^{a}
Nap	196.64 ± 8.94^{b}	7.54 ± 0.91^{a}
Art-Lum + Nap	174.30 ± 35.47^{b}	6.63 ± 1.34^{a}

Values are expressed as Mean ± SEM (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

The combined administration of Artemether-Lumefantrine and Naproxen significantly reduced ($p<0.05$) the Serum Total Protein in comparison to the control but did not significantly alter ($p>0.05$) the Albumin. The combination significantly decreased ($p<0.05$) the Globulin relative to the control (Table 3).

Table 3: Effects of Artemether-Lumefantrine and Naproxen combination on Total Protein, Albumin and Globulin in rat Serum

Group	**Total Protein (g/l)**	**Albumin (g/l)**	**Globulin (g/l)**
Control	428.81 ± 67.59^{b}	60.78 ± 1.13^{a}	368.04 ± 67.67^{b}
Art-Lum	177.24 ± 17.27^{a}	58.80 ± 8.28^{a}	118.48 ± 15.45^{a}
Nap	122.38 ± 19.21^{a}	67.15 ± 3.15^{a}	55.23 ± 21.60^{a}
Art-Lum + Nap	86.74 ± 7.45^{a}	57.28 ± 8.24^{a}	29.46 ± 1.83^{a}

Values are expressed as Mean ± SEM (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

The combined administration of Artemether-Lumefantrine and Naproxen did not significantly alter ($p>0.05$) the alkaline phosphatase activity in liver. The combination, when compared to the control, significantly decreased ($p<0.05$) the alkaline phosphatase activity in kidney but significantly increased ($p<0.05$) alkaline phosphatase activity in serum (Table 4).

Table 4: Effects of Artemether-Lumefantrine and Naproxen combination on Alkaline Phosphatase activities in rat tissues

	Specific Activities of Alkaline Phosphatase (IU/g protein) × 10^{-3}		
Group	**Liver**	**Kidney**	**Serum**
Control	171.44 ± 72.75^{a}	4090.23 ± 763.90^{b}	619.42 ± 88.33^{a}
Art-Lum	226.58 ± 65.21^{a}	1600.16 ± 573.68^{a}	2228.05 ± 595.64^{b}
Nap	157.41 ± 22.41^{a}	1730.24 ± 172.19^{a}	1646.26 ± 484.43^{ab}
Art-Lum + Nap	160.45 ± 31.97^{a}	2286.32 ± 393.76^{a}	2835.99 ± 546.06^{b}

Values are expressed as Mean ± SEM (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

The combined administration of Artemether-Lumefantrine and Naproxen did not significantly alter ($p>0.05$) the alanine aminotransferase activity in liver. The combination, compared to the control, significantly decreased ($p<0.05$) the alanine aminotransferase activity in kidney but significantly increased ($p<0.05$) alanine aminotransferase activity in serum relative to the control (Table 5).

Table 5: Effects of Artemether-Lumefantrine and Naproxen combination on Alanine Aminotransferase activities of rat tissues

	Specific Activities of Alanine Aminotransferase (IU/g protein)×10^{-3}		
Group	**Liver**	**Kidney**	**Serum**
Control	3444.41 ± 276.97^{a}	2273.21 ± 569.15^{b}	37.27 ± 7.91^{a}
Art-Lum	3355.96 ± 295.34^{a}	1348.15 ± 204.45^{ab}	92.81 ± 8.38^{a}
Nap	4575.57 ± 885.08^{a}	1123.13 ± 111.88^{a}	178.58 ± 22.59^{a}
Art-Lum + Nap	5450.11 ± 973.53^{a}	1199.45 ± 41.14^{a}	382.26 ± 80.60^{b}

Values are expressed as Mean ± SEM (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

The combined administration of Artemether-Lumefantrine and Naproxen did not significantly change ($p>0.05$) aspartate aminotransferase activity in liver and did not significantly alter ($p>0.05$) the aspartate aminotransferase activity in kidney but significantly increased ($p<0.05$) aspartate aminotransferase activity in serum compared to the control (Table 6).

Table 6: Effects of Artemether-Lumefantrine and Naproxen combination on Aspartate Aminotransferase activities in rat tissues

	Specific Activities of Aspartate Aminotransferase (IU/g protein)×10^{-3}		
Group	**Liver**	**Kidney**	**Serum**
Control	4099.89 ± 621.69^{a}	30775.94 ± 1818.68^{a}	187.25 ± 14.30^{a}
Art-Lum	3819.33 ± 377.99^{a}	27786.60 ± 2754.94^{a}	490.67 ± 68.28^{b}
Nap	4063.46 ± 247.61^{a}	27088.19 ± 2903.33^{a}	902.79 ± 48.97^{c}
Art-Lum + Nap	4798.15 ± 127.98^{a}	31763.56 ± 3409.50^{a}	972.58 ± 63.13^{c}

Values are expressed as Mean ± SEM (n=3). Values in each column with different alphabet superscripts are significantly different ($p<0.05$).

IV. Discussion

Changes in the organ-body weight ratio may be an indication of cell constriction or inflammation since the cells are the unit components of the organs. The constriction in the organ may occur as a result of loss of fluid from the organ due to damage, while increase in organ-body weight ratio may suggest inflammation [17]. The result from this study indicate that the liver neither constrict nor increase in size. Whereas, the independent administration of administration of the drugs did not bring about alteration of the kidney-body ratio, the combination of the drugs resulted in significant decrease in kidney-body weight ratio. This suggest constriction of the kidney due to the combined administration of Artemether-Lumefantrine and Naproxen.

Urea results from the catabolism of proteins and amino acids. The biosynthesis of urea is carried out exclusively by hepatic enzymes of urea cycle and it is majorly cleared from circulation by the kidneys. Consequently, kidney malfunction is associated with accumulation of urea in the blood [18]. Creatinine is formed from spontaneous cyclization of creatine and creatine phosphate, at a slow but constant rate and it is rapidly removed from the blood by the kidney [5]. Measurements of serum concentrations of creatinine, urea and uric acid are commonly used as indicators of kidney function and their conditions [18]. The combined administration of Artemether-Lumefantrine and Naproxen does not alter the urea level suggesting that the urea cycle was not affected by the administration. But creatinine, which is a more sensitive indicator of the functionality of the kidney, significantly increased by the independent and combined administration of the drugs. This suggest that the combined administration of Artemether-Lumefantrine and Naproxen could cause reduction in the excretory function of the kidney.

Albumin is synthesized primarily by the hepatic parenchymal cells. Its major function is the maintenance of osmotic pressure and also transport of compounds. Decreased concentration of albumin could be as a result of direct inhibition of synthesis by toxins [19]. Globulins are plasma protein that have protective function; as immunity component. These serum proteins are synthesized by the liver [20]. Both the independent and combined administration of Artemether-Lumefantrine and Naproxen did not alter the albumin concentration but resulted in decrease concentration of total protein and globulin concentration. This suggest that the administration of Artemether-Lumefantrine and Naproxen could bring about reduction in the ability of the liver to synthesize these macromolecules.

Tissue enzyme assay (such as AST, ALT and ALP) can indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by chemical agents [21].

Alkaline phosphatase is a membrane-bound enzyme and its activity is associated with membranes and cell surfaces of organs [22]. It is often assayed for, to assess the integrity of plasma membrane [23]. Alkaline phosphatase functions to liberate inorganic phosphate from an organic phosphate ester [24]. Both the independent and combined administration of Artemether-Lumefantrine and Naproxen had no significant effect

on the alkaline phosphatase activity of the liver but the significant decrease observed in the kidney with concomitant increase in the activity of alkaline phosphatase in the serum suggest damage to the membrane of kidney cells. Hence, compromised integrity of the kidney cells is suspected.

Aminotransferases catalyze the transfer of amino groups from one carbon skeleton to another and require the coenzyme pyridoxal phosphate [5]. Alanine aminotransferase is exclusively cytoplasmic while Aspartate aminotransferase exist both as mitochondrial and cytoplasmic forms [22]. The presence of elevated plasma levels of aminotransferases indicates damage to cells rich in these enzymes [5]. The combined administration of Artemether-Lumefantrine and Naproxen had no significant effect on the liver ALT but resulted in significant decrease in ALT activity of kidney with concomitant increase in the ALT activity in serum. Also, AST activity in the liver and the kidney was not affected by the combined administration but was significantly increased in serum. These suggest possible leakage or stimulation of ALT activity by the combined administration and alteration of the amino acid metabolism in the kidney.

V. Conclusion

The outcome of this research suggest that the combined administration of Artemether-Lumefantrine and Naproxen could cause alteration in the excretory function of the kidney. The synthetic power of the liver could also be affected but the kidney appears more affected. The combined administration of Artemether-Lumefantrine and Naproxen could result in damage to the kidney cells.

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