ARTEMISININ COMBINATION THERAPY ON SOME LIVER BIOCHEMICALS IN NIGERIAN MALE SUBJECTS

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ABSTRACT

Studies were carried out on dihydroartemisinin (DHA) administered as a monotherapeutic agent and in combination with piperaquine (PIP), and piperaquine with trimethoprim (PIP-TMP) on healthy Nigerian male volunteers. The effect of the monotherapeutic agent and in combination as artemisinin combination therapy (ACT) on the plasma levels of alanine aminotransferase (ALT) and aspartate aminotransfererase (AST) enzymes as well as bilirubin (BIL) were analyzed using the end point calorimetric method. The results have shown that the co-administration of DHA with PIP and PIP-TMP on the fourth day of study caused an increase in the mean plasma concentrations of ALT to 96.25% and 98.13% against 71.41% for DHA alone. There was no significant difference in the levels of conjugated bilirubin (CBIL) and total bilirubin (TBIL) when DHA was administered as a monotherapeutic agent and in combination with PIP-TMP. The increase in the level of ALT and AST suggest that DHA as a monotherapeutic agent have injurious effect on the liver cells and allows these enzymes to leak into the plasma. The addition of PIP and PIP-TMP aggravates this effect however the concomitant administration with therapeutic doses of herpatonic (HT) neutralizes the adverse effect

INTRODUCTION

Malaria is a debilitating disease and has remained a vexatious issue since the dawn of history. The disease is on the increase in Nigeria and other Sub-Saharan areas of Africa, carrying along with it high morbidity and mortality (Coker *et al*., 2001, Adepoju-Bello and Ogbeche, 2003). Malaria causes significant economic loses, and can reduce gross domestic products (GDP) by as much as 1.3% in countries with high levels of transmission. The health cost of malaria includes both private and public expenditures on prevention and treatment. In some heavy burden countries, WHO economic impact report of 2010, showed that the disease account for up to 40% of public health expenditures, 50% of inpatient hospital admissions and 60% of outpatient health clinic visits. Malaria disproportionately affects people who cannot afford treatment or have limited access to health care, trapping families and communities in a downward spiral of poverty (WHO, 2010).

Plasmodium, a unicellular eukaryotic cell of the protozoa phylum is the fatal parasite responsible for malaria disease. The pathogen is transmitted by the Anopheles mosquito. In the human body, the parasite multiply in the liver, brain, lungs, kidneys, placenta and other tissues (David and Peter 2004; Adams *et al*., 2002; Alkawe *et al*., 1988)

Control measures of malaria include preventive (which involves control of the vector of the malaria parasite by indoor spraying with residual insecticides, sleeping under Long Lasting Insecticide-impregnated nets (LLINs), the maintenance of good sanitary conditions and treatment of the disease with the use of drugs (Habluetze *et al.,* 1999; Brugha *et al*., 1999). Drug treatment of the malaria has so far not been a complete success story, because of the complicity of the life cycle of the protozoa (*Plasmodium*) both in human and in the mosquitoe

vector, and the development of resistance by Plasmodium (Foote *et al*., 1990; Schellenberg, *et al.,* 1999; Ansel *et al*., 2002; Amed *et al.,* 2003). The emergence of resistance to chloroquine (CQ) and other antimalarials has led to the search and development of newer drugs including artemisinin and its derivatives, (Sanjeev *et al*., 2004). Artemisinin and its derivatives have a short elimination half and this confers the theoretical advantage that selection for drug resistant parasite is less likely, however to avoid the higher risk of recrudescence observed when they are used in monotherapeutic regimens, artemisinins are used in combination with other antimalarials with relatively longer elimination half life. This combination is known as Artmisinin Combination Therapy (ACT) and has been adopted as the front line drugs for the treatment of uncomplicated malaria in Nigeria (Giao, 2001; Unnati and Charu, 2002; Olliaro and Taylor,2002; Garner and Graves, 2005; Davis *et al*., 2005).

Methionine and choline the chemical substances present in neurtosec the herpatonic (HT) used in the study, are known to be responsible for the integrity of liver cells, these together in combination protects the liver from fatty infiltration by detoxifying certain exogenous poisons (Garner and Graves, 2005). Artemisinins are considered to have high safety margins, however, they may be toxic under certain conditions such as when used in high concentrations and in ACT (Udobre *et al*., 2009; Etim *et al*., 2012). The present study is aimed at investigating the effect of dihyroartemisinin as a monotherapeutic agent and in combination as ACTs on the liver functions and the influence of half and therapeutic doses of HT on such effects.

MATERIALS AND METHODS

The study was conducted between October and December 2011 at the Health Centre of the University of Uyo, after approval by the Ethics committee of the University.

Recruitment of Subjects

Eighty healthy male volunteer subjects were identified through personal contact from among students, and staff of the University of Uyo community. Written informed consent to participate in the study was sought and obtained from the volunteers. They were physically fit with no history of malaria infection or treatment with any antimalaria drug or any other medication, in two weeks before commencement of the study. Volunteers who took any form of tobacco or cigarettes were also excluded. They were also screened and found to be free from any chronic liver and kidney diseases by assessing microscopically, the presence of protein and creatinine in urine. The volunteers were randomly divided into ten groups with eight volunteers per group.

Vital signs

Body temperature, body weight not less than 60kg and blood pressure of each volunteer were recorded before drug administration.

Drugs / Chemical

The drugs used in this study were dihydroartemisinin 60mg table (alaxin manufactured by Bliss GVS India); P-alaxine, containing DHA 40mg and piperaquine phosphate (PIP) 320mg manufactured by Bliss GVS India; artecxin, containing DHA- 32mg, piperaquine phosphate-320mg and trimethoprim (TRM) 90mg, manufacture for Medicare Pharma Ltd. Sango Otta Nigeria; neutrosec syrup the hepatotonic HT containing methionine 100mg, choline dihydrogen citrate 100mg in 15ml of solution and other vitamins,made by Tablets (India) Ltd., and marketed by Fidson Healthcare Ltd. Nigeria. All the drugs were obtained direct from the manufacturer's representatives here in Uyo and they were all less than one year from the date of manufacture. The chemicals were freshly prepared as explained in Randox manual for the various substances.

Study Design

An open single centre study involving all the subjects was carried out. After an overnight fast and a light breakfast at 8.00 am, the subjects were divided into ten groups with eight volunteers

in each group. The entire subjects were to abstain from any other medication, alcohol and cigarettes within the period of the studies. They were also to eat the same types of snacks and food within the period of study.

Administration of drugs to volunteer:
Each member in the groups received adult doses of the following drugs, swallowed with 500ml of water:

Group A: 4 tablets of alaxine only.
Group B: 4 tablet of alaxine with 15ml of neutrosec.
Group C: 4 tablets of alaxine with 30ml of neutrosec.
Group D: 3 tablet of P-alaxin only daily.
Group E: 3 tablets of P-alaxine with 15ml of neutrosec.
Group F: 3 tablets of P-alaxine with 30ml of neutrosec.
Group G: 4 caplets of artecxin only.
Group H: 4 tablets of artecxin with 15ml of neutrosec.
Group I: 4 tablets of artecxin with 30ml of neutrosec.
Group J: Control took no drugs.

Notes
i.) Alaxin was taken daily for three days.
ii.) P-alaxin was taken daily for three days
iii.) Artecxin was taken daily for two days
iv.) The 15ml and 30ml neutrosec were taken in two doses daily for seven days.
v.) The dosing was as recommended by the manufacturers and was in conformity with WHO standard for the treatment of uncomplicated malaria.

Collection of Blood and Preparation of Plasma
Precisely 5.0ml of blood was collected from each volunteer subject before drug administration on the first day. After administration of the drugs, 5.0ml of blood was collected from each of the volunteers between 8.00am and 10.0 am on the 2^{nd}, 4^{th}, 7^{th} and 14^{th} days. The fresh blood was placed in heparinzed tubes and allowed to stand for 5 minutes for equilibration at room temperature. The conditioned blood sample was put in a clean centrifuge tube and spun at 5,000 rpm for 20 minutes in a centrifuge machine (MSE England) to separate the plasma from the cells. The plasma sample was aspirated, transferred into a specimen bottle, and stored at -15^{o}C for a period not exceeding 24 hour.

Quantitative Determination of AST, ALT and BIL
The prepared plasma samples obtained from the blood of volunteers were analyzed. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured using the end point calorimetric method as explained in Randox manual (Retman and Frankel, 1957). The absorbance of the test solution was measured using an UV-vis NIR spectrophotometer with wavelength of 540nm at 37^{o}C against sample blank. The enzyme activity is gives in IUL^{-1}. Bilirubin was measured using the end point calorimetric method. Total Bilirubin (TBIL) was measured at a wavelength of 580nm at 25^{o}C against sample blank, while Direct Bilirubin (DBIL) was estimated at wavelength of 550nm at 25^{o}C against sample blank. Amount of BIL is measures in µmol/L or mg/dl.

Statistical Analysis
The values obtained were expressed as percentage increase (+) or decrease (-) of the mean value of the blank. The data obtained were expressed as mean ± SD. Student T-test was used to assess statistical significance and values of $P<0.05$, were considered to be significant.

RESULTS AND DISCUSSION

The increase or decrease of the various parameters (liver biochemicals) was expressed as percentage increase or decrease relative to the mean of the blank samples in each group. The

control was to check the normal physiological variation of the biochemicals in volunteers who did not take any drug. The HT were administered at therapeutic doses 30ml/24hrs; and half the therapeutic doses 15ml/24hrs. On the 2nd and 4th days of measurement respectively DHA alone caused an average increase in the level of activity of AST by 32.83% and 40.89% above the mean of the blank values. The normal physiological variation in levels of ALT was ±6.68%. On the same days when DHA was used alone, and in combination with PIP, the mean values increased to 40.33% and 66.67% respectively, however the addition of TMP did not cause a significant change in the values of DHA with PIP, (Table 1).

Table 1: Effect of dihydroartemisinin alone and dihydroartemisinin plus piperaquine on the plasma levels of Alanine aminotransferase (ALT).

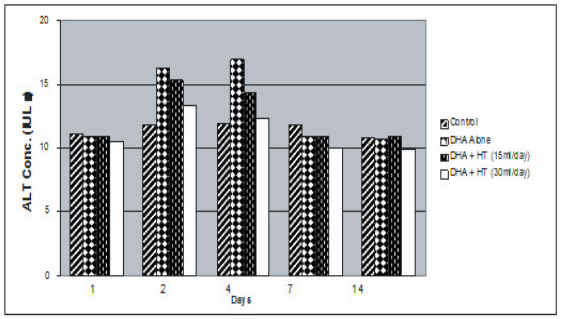
Days	DHA Alone		DHA + PIP		Control	
	ALT (IUL^{-1})	ALT (%)	ALT (IUL^{-1})	ALT (%)	ALT (IUL^{-1})	ALT (%)
1	9.7±1.37	-	8.0±3.54	-	11.17±3.11	-
2	16.2±2.26	67.0	15.7±2.11	96.25	11.89±2.01	6.44
4	16.9±2.17	74.0	15.2±3.01	90.00	12.01±2.21	7.52
7	10.76±3.31	11.0	10.1±2.72	26.25	11.87±3.11	6.26
14	10.1±2.11	4.1	8.4±3.21	5.00	10.21±2.53	-8.59

During the period of studies, the physiological variations in the activity of AST in the control group was ± 8.59%. On the 2nd and 4th days of measurements DHA alone caused an increase of 67.0% and 74.0% in the mean levels of AST respectively, while the addition of PIP to DHA caused an increase of 96.25% and 98.36 respectively. The addition of TMP to DHA and PIP did not cause any significant variation in the level of AST, (Table 2).

Table 2: The effect of dihydroartemisinin alone and dihydroartemisinin plus piperaquine on the levels of plasma aspartate aminotransferase (AST)

Days	Dihydroartemisinin Alone		DIH + PIP		Control	
	AST (IUL^{-1})	AST (%)	AST(IUL^{-1})	AST(%)	AST(IUL^{-1})	AST (%)
1	33.5 ± 3..91	-	30.0 ± 4.01	-	28.04± 3.75	-
2	44.5 ± 4.30	32.83	41.8±2.48	39.33	29.07±3.48	3.56
4	47.2 ± 3.80	40.89	50.0±6.01	66.66	27.10±4.11	-3.35
7	34.2 ± 3.85	20.89	24.5±2.58	-18.33	26.98± 2.11	3.78

All the values recorded for the liver biochemicals were within normal physiological levels, but the increase were statistically significant. For normal subjects, the use of the ACTs is quite in order, but for patients with liver problem or the elderly, the drug combination should be administered with caution and liver function test should be performed before drug administration. The drug combinations could be co-administered with a hepatotonic (HT) such as neutrosec, since it causes a dose dependent decrease in the activity of plasma AST and ALT, (Figs. 1 and 2).



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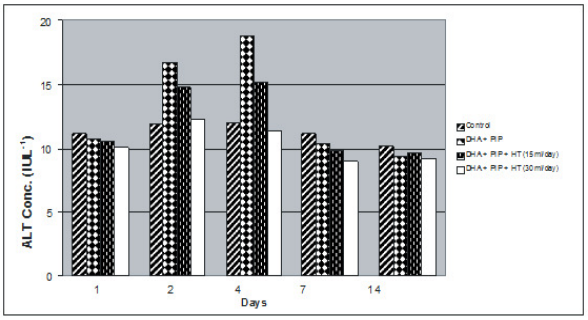

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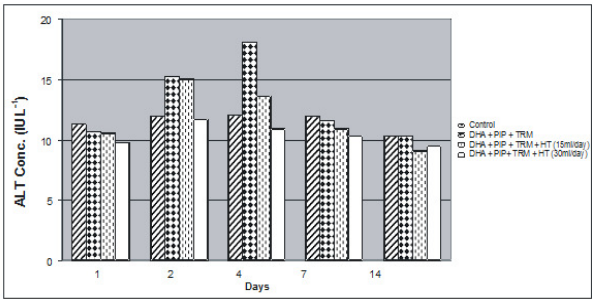
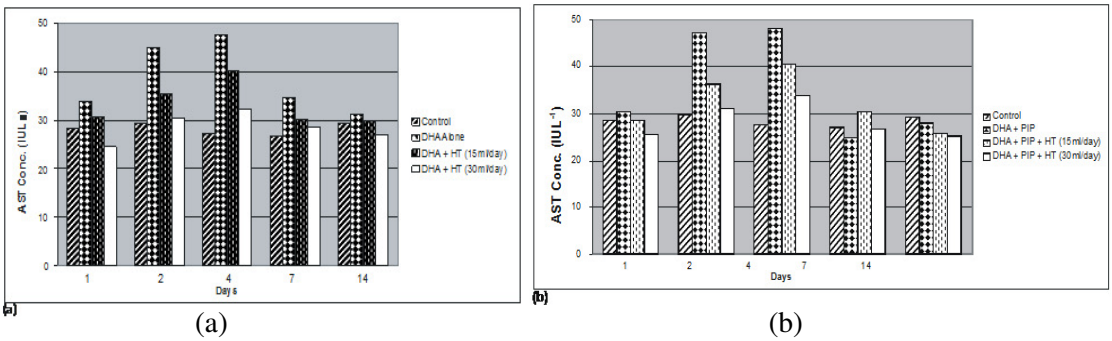


Figure 1: The effect of half (15ml/day) and the therapeutic doses (30ml/day) of hepatotonic (HT) on the activity of plasma alanine aminotransferase (ALT) during coadministration with (a) dihydroartemisinin (DHA) (b) DHA with piperaquine (PIP) (c) DHA with PIP and trimethoprime (TRM). Data are expressed as mean. n = 8.



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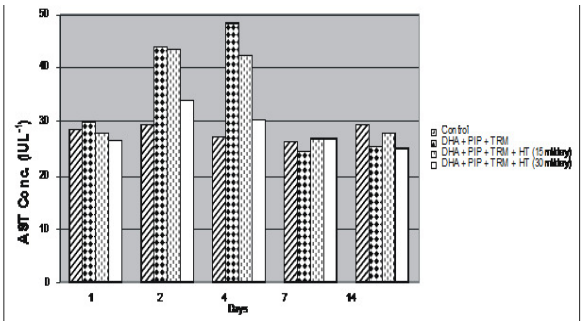


Figure 2: The effect of half (15ml/day) and the therapeutic doses (30ml/day) of hepatotonic (HT) on the activity of plasma aspartate aminotransferase (AST) during coadministration with (a) dihydroartemisinin (DHA) (b) DHA with piperaquine (PIP) (c) DHA with PIP and trimethoprime (TRM).Data are expressed as mean. n = 8.

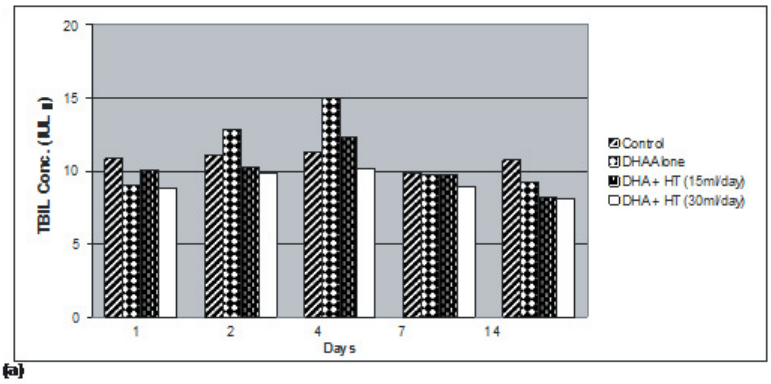
The normal physiological levels of conjugated bilirubin (CBIL) is up to 4.3µmol/L, and that of total bilirubin (TBIL) is 2-17 µmol/L (Marshall and Bangert, 2009). The normal variation in the level of TBIL in the control group was ± 4.5 % of the mean during the period of study. DHA alone caused an increase of 63.87% in the level of TBIL and 40.85% in the level of CBIL, on the 4th day of the studies. On this same day, the addition of PIP and TRM to DHA as ACT did not cause significant changes in the levels of CBIL and TBIL ($P<0.05$) (Tables 3 and 4).

Table 3: Effect of dihydroartemisinin alone and dihydroartemisinin plus piperaquine on the plasma levels of total bilirubin (TBIL)

Days	Dihydroartemisinin		Dih Plus Pip		Control	
	TBIL (IUL^{-1})	TBIL (%)	TBIL (IUL^{-1})	TBIL (%)	TBIL (IUL^{-1})	TBIL (%)
1	9.05±0.73	-	12.95±1.03	-	10.75±1.31	-
2	10.85±0.78	18.88	15.2±1.45	17.88	10.99±1.13	2.23
4	14.83±1.11	63.87	19.75±1.47	52.50	11.01±0.30	2.42
7	9.6±0.65	6.07	12.5±1.0	-3.47	9.8±1.21	-0.88
14	9.10±0.21	0.55	12.57±1.3	-2.93	10.72±0.40	-0.27

High levels of CBIL indicate that bile is not being properly excreted, therefore obstruction may be present in the bile duct or gall bladder. High levels of unconjugated bilirubin is an indication that too much hemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving (Craziadei, 2011). The results obtained from this study have shown that the administration of DHA as monotherapeutic agent and as ACT caused a significant increase in the level of both CBIL and TBIL ($P<0.05$). These values are of clinical importance when deciding on a combination regimen for patients who are anemic or those with liver problems. However, the co-administration of DHA with therapeutic doses of HT restored the levels of CBIL and TBIL to normal, (Figs 3 and 4).

(a)



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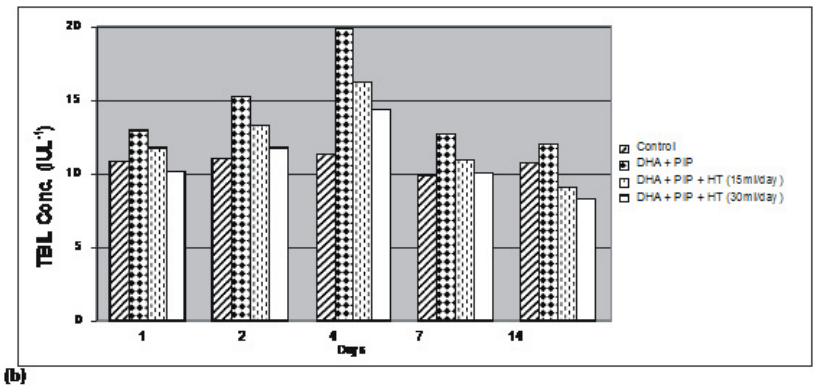

Figure 3: Effect of half (15ml/day) and therapeutic dose (30ml/day) of hepatotonic (HT) on the activity of plasma aspertate aminotransferase (AST) during coadministration with (a) DHA (b) DHA + PIP (c) DHA + PIP + TRM.

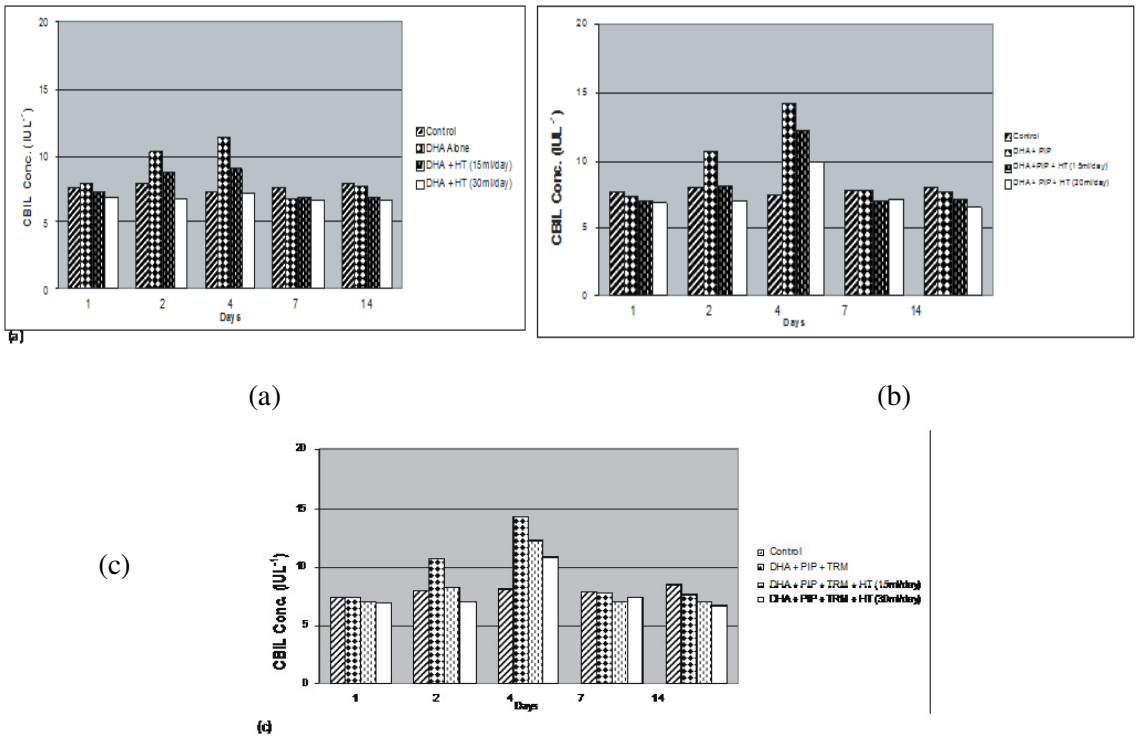

Figure 4: Effect of half (15ml/day) and therapeutic doses (30ml/day) of hepatotinic (HT) on the level of conjugated bilirubin (CBIL) in plasma during coadministration with (a) DHA (b) DHA + PIP (c) DHA + PIP + TRM. Data are expressed as mean. n = 8

CONCLUSION AND RECOMMENDATION

From the results of this study, it is apparent that the administration of therapeutic doses of DHA as a monotherapeutic agent may be toxic to the liver by casing an elevation in the plasma level of hepatic enzymes and bilirubin . The co-administration of DHA with its partner drugs as ACT aggravates there effects by increasing the activity of AST and ALT. However, it is recommended that the pre-administration, co-administration and post-administration of the ACTs with therapeutic doses of hepatotonic should always be employed to cushion the effects.

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