**Physiology and Pharmacology**

**Physiol Pharmacol 20 (2016) 287-295** **www.phypha.ir/ppj**

Original Article

# Melatonin and alpha lipoic acid as possible therapies for lopinavir/ritonavir-induced hepatotoxicity in albino rats

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## Abstract

**Introduction:** The use of lopinavir/ritonavir (LPV/r) has decreased morbidity and mortality due to human immunodeficiency virus (HIV); however its use could be impaired by hepatotoxicity. Therefore, this study was designed to investigate the effects of melatonin (MT) and alpha lipoic acid (ALA) on LPV/r-induced hepatotoxicity in male albino rats.

**Methods:** Rats were divided into groups and treated with MT (10 mg/kg/day), ALA (10 mg/kg/day) and LPV/r (22.9/5.71, 45.6/11.4 and 91.2/22.9 mg/kg/day) for 60 days respectively. Rats were pretreated with MT (10 mg/kg), ALA (10 mg/kg) and combined doses of ALA and MT prior to treatment with LPV/r (22.9/5.71, 45.6/11.4 and 91.2/22.9 mg/kg/day) for 60 days. Rats were sacrificed and serum was collected and evaluated for liver enzymes. The liver was harvested and evaluated for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) levels.

**Results:** Significant ($P$<0.05) decreases in baseline serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and liver MDA levels with increases in liver SOD, CAT and GSH levels were obtained in MT and ALA treated animals when compared to control. On the contrary, significant ($P$<0.05) and dose dependent increases in serum AST, ALT, ALP and liver MDA levels with decreases in liver SOD, CAT and GSH levels were obtained in LPV/r treated rats when compared to placebo control. However, LPV/r-induced changes in the above parameters were attenuated in MT and ALA pretreated rats. Attenuations were significantly ($P$<0.05) different in rats pretreated with combined doses of MT and ALA when compared to their individual doses.

**Conclusion:** Results of this study showed that MT and ALA could be used for the treatment of LPV/r associated hepatotoxicity.

**Keywords:**
Liver;
Toxicity;
Lopinavir/ritonavir;
Antioxidants;
Pretreatment;
Rats

**Received:** 2 Aug 2016
**Accepted:** 8 Oct 2016

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## Introduction

Human immunodeficiency virus (HIV), the virus that causes AIDS (acquired immunodeficiency syndrome) has become one of the world's most serious health and development challenges. Current management of HIV requires at least three classes of antiretroviral drugs, this combination is known as highly active antiretroviral therapy (HAART). Lopinavir is a

protease inhibitor used as a vital component of HAART and is usually co-formulated with ritonavir (Kumar et al., 1999). The introduction of LPV/r (lopinavir/ritonavir) in combination with other antiretroviral drugs has decreased mortality, morbidity and prolonged life expectancy of people living with HIV. However, the use of LPV/r in the management of HIV could be associated with some toxicities including hepatotoxicity (Sulkowski, 2004; Almond et al., 2004; Hughes et al., 2011). Clinical trials reported 10% of LPV/r associated hepatotoxicity characterized by grade 3 and 4 increases in aminotransferases (Kemmer et al., 2000; Chihrin et al., 2004). Hepatitis, hepatic failure and death could be associated with the use of LPV/r and there may be an increased risk for aminotransferase elevations in patients with preexisting liver disease (Sulkowski, 2004). Also, studies have reported structural alterations in the liver of LPV/r treated animals (Van Gend, 2008). Hepatotoxicity due to treatment with LPV/r-induced hepatotoxicity could be associated with oxidative stress due to reported mitochondria damage, oxidative radical production and the depletion of antioxidants in animal studies (Zaera et al., 2001; Chandra et al., 2009; Touzet and Philips, 2010).

Melatonin (MT) and its major hepatic metabolites are antioxidants, which can reduce oxidative stress via scavenging of oxidative radicals and the regeneration of other antioxidants (Tan et al., 2001; Tan et al., 2007). MT has a plethora of significant actions which include oncostatic effect, immune system stimulation and anti-inflammatory functions (Blask et al., 2002). Also, it has hepatoprotective activity as evidenced by the inhibition of lipopolysaccharide (LPS)-induced hepatotoxicity in endotoxemic rats (Sewerynek et al., 1995; Crespo et al., 1999). It has also been found that MT has protective effect against immunological liver injury induced by *Bacillus Calmette-Guerin* and LPS (Wang et al., 2004) In addition; MT has protective effect against LPS-induced liver damage in galactosamine sensitized mice (Wang et al., 1997).

Alpha lipoic acid (ALA) is a cofactor of α-ketoacid dehydrogenase complexes and plays a fundamental role in fuel metabolism (Siti et al., 2008). It has been found that ALA affects cellular metabolic processes, alters redox status of cells and interacts with thiols and other antioxidants (Packer et al., 2001). It is an amphiphilic antioxidant that quenches reactive oxygen species, chelates metal ions and reduces the oxidized forms of other antioxidants. It has anti-inflammatory effect and can inhibit the release of inflammatory cytokines and other inflammatory mediators (Henriksen et al., 2006; Heibashy et al., 2013). It can inhibit xenobiotic-induced liver toxicity as reported in adriamycin-induced hepatotoxicity in rats (Anandakumar et al., 2007) and restored hepatic function in chloroquine intoxicated rats (Pari and Murugavel, 2004). In addition, it ameliorated aflatoxin B1-induced excess production of lipid peroxides and maintained intracellular antioxidant status in the liver (Li et al., 2014). Furthermore, studies have reported synergistic activity with concurrent use of MT and ALA (Mukherjee et al., 2011). Therefore, the present study was designed to investigate the effects of MT and ALA on LPV/r-induced hepatotoxicity in albino rats.

# Materials and methods

### Animals

Eighty five healthy adult male albino rats were used for this study. The rats were supplied by the animal house of the University of Port Harcourt, Choba, Rivers State. The rats were housed in individual cages at 21±2 °C, 40–60% relative humidity and exposed to a 12-h light–dark cycle, with the light cycle coinciding with daylight hours. The rats were allowed free access to food and water ad libitum.

### Drugs

Lopinavir/ritonavir (LPV/r) (Myland Laboratories Limited India), melatonin and alpha lipoic acid (AO Pharm Import and Export Co Ltd China) were used for this study. All other chemicals used for this study were of analytical grade. LPV/r (22.9/5.71, 45.6/11.4 and 91.2/22.9 mg/kg) (Hull et al., 2009), MT 10 mg/kg and ALA 10 mg/kg (Ali, 2013; Bilginoğlu et al., 2014) were used for this study. ALA was dissolved in water (Shagirtha et al., 2011). LPV/r was dissolved in 1% ethanol (Reyskens et al., 2013), while MT was dissolve in 1% ethanol and diluted with normal saline (Kaplan et al., 2009).

### Experimental design

Rats used for this study were divided into 6 groups (A-F). Group A served as the control and was divided into two sub-groups, A1 and A2 of 5 rats each. Rats in group A1 (placebo control) and A2 (solvent control)

were orally treated with normal saline and 1% ethanol for 60 days respectively. Groups B-F contained 15 rats each which were divided into 3 sub-groups of 5 rats each. Rats in group B were orally treated with 10 mg/kg/day of ALA and combined doses of MT and ALA for 60 days. Rats in group C were orally treated with 22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg/day of LPV/r for 60 days. Groups D-F were pretreated with 10 mg/kg/day of MT, 10 mg/kg/day of ALA and combined doses of MT and ALA prior to oral treatment with 22.9/5.71, 3 45.6/11.4 and 91.4/22.9 mg/kg/day of LPV/r, for 60 days respectively.

**Collection of sample for analysis**

Animals were sacrificed using diethyl ether and blood samples were collected via cardiac puncture. The blood samples were allowed to clot and centrifuged at 1200 rpm for 15 min and serum separated for the evaluation of liver function parameters. Liver was harvested via dissection and washed in an ice cold 1.15% potassium chloride solution. Liver was homogenized in 0.1 M phosphate buffer (pH 7.2) then centrifuged at 2500 rpm speed for 15 min. The supernatant was decanted and used for the evaluation of oxidative stress indices.

**Evaluation of serum liver function parameters and liver oxidative stress indices**

Aspartate aminotransferase and alanine aminotransferase were evaluated as reported by Reitman and Frankel, 1975. Alkaline phosphatase (ALP) was evaluated as reported by Babson et al., 1966. Liver malondialdehyde (MDA) was evaluated as reported by Buege and Aust, 1978 while superoxide dismutase (SOD) was evaluated according to the method of Sun and Zigma, 1978. Glutathione (GSH) analyzed according to Sedlak and Lindsay, 1968 while catalase (CAT) was evaluated as reported by Sinha et al., 1972.

**Statistical analysis**

Data was analyzed using one way analysis of variance. Results are expressed as mean ± standard error of mean (SEM). Statistical significance set was at $P<0.05$.

# Results

In this study, rats treated with individual doses of MT and ALA showed significant ($P<0.05$) decreases in baseline serum AST, ALT and ALP levels when compared to control. However, most pronounced decreases in baseline serum AST, ALT and ALP levels were obtained in rats co-administered with MT and ALA which were significantly ($P<0.05$) different when compared to their individual doses (Table1). Baseline MDA levels were significantly ($P<0.05$) decreased while SOD, CAT and GSH levels were significantly ($P<0.05$) increased in rats treated with individual doses of MT and ALA when compared to control. Interestingly, effects on MDA, SOD, CAT and GSH were most pronounced with concurrent use of MT and ALA and were significantly ($P<0.05$) different when compared to effects of treatments with their individual doses (Table1). Furthermore, liver MDA levels were increased while SOD, CAT and GSH levels were decreased significantly ($P<0.05$) and in a dose-dependent manner in rats treated with 22.9/5.71-91.2/22.9 mg/kg/day of LPV/r for 60 days when compared to control (Table 2). However,

**Table 1:** Effects of treatments with melatonin and alpha lipoic acid on baseline serum liver function parameters and oxidative stress indices of albino rats.

| DOSE | ALP (U/L) | AST(U/L) | ALT(U/L) | MDA nmole/mg protein | GSH µmole/mg protein | CAT U/mg protein | SOD U/mg protein |
|---|---|---|---|---|---|---|---|
| **Control** | 36.9 ± 2.60 | 36.1 ± 1.63 | 37.5 ± 2.39 | 0.76 ± 0.04 | 15.5 ± 0.55 | 15.2 ± 0.01 | 21.9 ± 0.65 |
| MT | 24.0 ± 1.62 | 22.7 ± 1.20* | 24.1 ± 1.34* | 0.50 ± 0.62* | 20.4 ± 0.49* | 25.6 ± 0.70* | 30.0 ± 2.33* |
| LA | 26.6 ± 1.78 | 25.4 ± 1.97* | 26.9 ± 1.81 | 0.54 ± 0.08* | 21.9 ± 0.49* | 23.6 ± 0.72* | 28.3 ± 1.19* |
| MT+LA | 15.5 ± 0.86** | 18.2 ± 0.68** | 18.9 ± 0.57** | 0.30 ± 0.03** | 32.2 ± 2.44** | 40.4 ± 3.33** | 40.5 ± 3.04** |

MT=Melatonin. ALA= Alpha lipoic acid. n=5. Results are expressed as mean ± SEM * Significant ($P<0.05$) difference when compared to control. ** Significant ($P<0.05$) difference when compared to treatments with individual doses of MT and ALA

**Table 2:** Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced serum levels of aminotransferases and alkaline phosphatase in male albino rats.

| Serum aspartate aminotransferase (U/L) | | | | |
|---|---|---|---|---|
| **Dose (mg/kg)** | **LPV/r** | **MT+LPV/r** | **ALA+LPV/r** | **MT+ALA+LPV/r** |
| **Control** | 36.13 ± 2.71 | 36.13 ± 2.71 | 36.13 ± 2.71 | 36.13 ± 2.71 |
| 22.9/5.71 | 66.43 ± 4.90 | 35.40 ± 1.44* | 37.55 ± 2.46* | 32.93 ± 1.42* |
| 45.6/11.4 | 86.30 ± 4.84 | 40.15 ± 2.75* | 41.73 ± 2.51* | 35.78 ± 2.47* |
| 91.2/22.9 | 101.1 ± 6.80 | 49.85 ± 3.42* | 52.18 ± 3.34* | 37.10 ± 3.89** |
| **Serum alkaline phosphatase (U/L)** | | | | |
| **Control** | 36.93 ± 2.60 | 36.93 ± 2.60 | 36.93 ± 2.60 | 36.93 ± 2.60 |
| 22.9/5.71 | 76.08 ± 4.00 | 39.28 ± 1.20* | 39.03 ± 2.34* | 32.18 ± 1.38* |
| 45.6/11.4 | 80.70 ± 3.74 | 48.78 ± 2.90* | 46.93 ± 2.96* | 35.28 ± 3.49** |
| 91.2/22.9 | 94.15 ± 4.12 | 51.08 ± 3.80* | 54.95 ± 3.04* | 39.10 ± 2.51** |
| **Serum alanine aminotransferase (U/L)** | | | | |
| **Control** | 37.53 ± 2.39 | 37.53 ± 2.39 | 37.53 ± 2.39 | 37.53 ± 2.39 |
| 22.9/5.71 | 79.88 ± 3.47 | 38.90 ± 2.49* | 43.33 ± 2.40* | 30.03 ± 1.42* |
| 45.6/11.4 | 86.78 ± 3.09 | 47.03 ± 2.28* | 45.00 ±2 .59* | 32.73 ± 1.26** |
| 91.2/22.9 | 92.33 ± 4.71 | 50.33 ± 3.44* | 53.55 ± 3.18* | 34.05 ± 2.49** |

MT = Melatonin. ALA= Alpha lipoic acid. n=5. Results are expressed as mean ± SEM * Significant ($P$<0.05) difference when compared to treatment with LPV/r. ** Significant ($P$<0.05) difference when compared to pretreatments with individual doses of MT and ALA

supplementations with individual doses of MT and ALA prior to treatment with 22.9/5.71-91.2/22.9 mg/kg/day of LPV/r produced significant ($P$<0.05) decreases in serum levels of AST, ALT and ALP when compared to treatment with LPV/r. Further and significant ($P$<0.05) decreases in serum AST, ALT and ALP levels were obtained in rats supplemented concurrently with MT and ALA when compared to supplementation with their individual doses (Table 2). Furthermore, liver MDA levels were increased while SOD, CAT and GSH levels were decreased significantly ($P$<0.05) and in a dose-dependent manner in rats treated with 22.9/5.71-91.2/22.9 mg/kg/day of LPV/r for 60 days when compared to control. However, this study obtained decreases in MDA levels with increases in SOD, CAT and GSH levels in rats pretreated with individual doses of MT and ALA prior to treatment with 22.9/5.71-91.2/22.9 mg/kg/day of LPV/r. The effects on MDA, SOD, CAT and GSH were significantly ($P$<0.05) different when compared to LPV/r treated rats. Interestingly, pretreatment with combined doses of MT and ALA further decreased MDA levels while SOD, CAT and GSH levels were increased. The effects on MDA, SOD, CAT and GSH levels in rats pretreated with combined doses of MT and ALA were significantly ($P$<0.05) different when compared to their individual doses (Table 3 and 4).

# Discussion

The liver functions in transforming and detoxifying drugs and metabolites. It also produces different types of plasma proteins such as albumin, which are delivered into the blood, as well as metabolites that are constituents of the bile (Sasse et al., 1992; Arias et al., 1997). The constant involvement of the liver in drug biotransformation could lead to hepatotoxicity (Woodward et al., 2009; An et al., 2011). Oxidative stress produced by free radicals has been implicated in the pathogenesis of drug-induced hepatotoxicity (Stehbens, 2003). Therefore, this study evaluated the effects of MT and ALA on LPV/r- induced hepatotoxicity in male albino rats. The present study observed decreases in baseline AST, ALT, ALP and MDA levels with increases in SOD, GSH and CAT

**Table 3:** Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced liver levels of malondialdehyde and superoxide dismutase in male albino rats.

| Liver malondialdehyde (nmol/mg protein) | | | | |
|---|---|---|---|---|
| **Dose (mg/kg)** | **LPV/r** | **MT+LPV/r** | **ALA+LPV/r** | **MT+ALA+LPV/r** |
| **Control** | 0.86 ± 0.04 | 0.86 ± 0.04 | 0.86 ± 0.04 | 0.86 ± 0.04 |
| 22.9/5.71 | 2.38 ± 0.01 | 0.87 ± 0.01* | 0.93 ± 0.02* | 0.63 ± 0.01** |
| 45.6/11.4 | 3.90 ± 0.01 | 0.90 ± 0.08* | 0.13 ± 0.01* | 0.67 ± 0.08** |
| 91.2/22.9 | 5.65 ± 0.05 | 1.23 ± 0.08* | 1.48 ± 0.07* | 0.70 ± 0.03** |
| **Liver superoxide dismutase (U/mg protein)** | | | | |
| **Control** | 15.21 ± 1.01 | 15.21 ± 1.01 | 15.21 ± 1.01 | 15.21 ± 1.01 |
| 22.9/5.71 | 8.98 ± 0.04 | 13.20 ± 0.11* | 12.24 ± 0.12* | 16.42 ± 0.33* |
| 45.6/11.4 | 6.45 ± 0.02 | 11.21 ± 0.18* | 9.27± 0.37* | 16.17 ± 0.13** |
| 91.2/22.9 | 3.89 ± 0.08 | 8.03 ± 0.05* | 7.02 ± 0.05* | 14.13 ± 1.56** |

MT= Melatonin. ALA = Alpha lipoic acid. n=5. Results are expressed as mean ± SEM * Significant ($P$<0.05) difference when compared to treatment with LPV/r. ** Significant ($P$<0.05) difference when compared to pretreatments with individual doses of MT and ALA

**Table 4:** Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced liver levels of catalase and glutathione in male albino rats.

| Liver catalase (U/mg protein) | | | | |
|---|---|---|---|---|
| **Dose (mg/kg)** | **LPV/r** | **MT+LPV/r** | **ALA+LPV/r** | **MT+ALA+LPV/r** |
| **Control** | 21.88 ± 1.65 | 21.88 ± 1.65 | 21.88 ± 1.65 | 21.88 ± 1.65 |
| 22.9/5.71 | 10.40 ± 0.55 | 18.80 ± 1.32* | 17.40 ± 1.20* | 23.45 ± 1.21** |
| 45.6/11.4 | 7.60 ± 0.01 | 17.85 ± 1.21* | 16.98 ± 1.15* | 22.23 ± 1.16** |
| 91.2/22.9 | 5.05 ± 0.05 | 14.75 ± 0.93* | 12.68 ± 0.18* | 24.05 ± 1.25** |
| **Liver glutathione (µmol/mg protein)** | | | | |
| **Control** | 15.48 ± 0.35 | 15.48 ± 0.35 | 15.48 ± 0.35 | 15.48 ± 0.35 |
| 22.9/5.71 | 6.25 ± 0.09 | 14.95 ± 0.15* | 14.55 ± 1.25* | 15.98 ± 1.02* |
| 45.6/11.4 | 4.37 ± 0.01 | 11.08 ± 0.23* | 10..88 ± 0.75* | 15.51 ± 1.05** |
| 91.2/22.9 | 3.51 ± 0.02 | 7.30 ± 0.08* | 7.23 ± 0.32* | 14.80 ± 0.91** |

MT= Melatonin. ALA = Alpha lipoic acid. n=5. Results are expressed as mean ± SEM * Significant ($P$<0.05) difference when compared treatment with LPV/r. ** Significant ($P$<0.05) difference when compared to pretreatments with individual doses of MT and ALA

levels in rats treated with MT and ALA. These observations are in agreement with previous reports (Bilginoğlu et al., 2014). The effects on the above parameters were most pronounced in rats treated concurrently with MT and ALA. On the contrary, dose-dependent increases in serum AST, ALT, ALP and liver MDA levels with decreases in SOD, GSH and CAT levels were obtained in rats treated with LPV/r. These findings are consistent with some reported observations (Kontorinis and Dieterich, 2003; Sulkowski, 2003; Chai et al., 2005; Chandra et al., 2009; Deng et al., 2010). AST, ALT and ALP are considered as markers of hepatocellular injury; therefore, increases in their levels in LPV/r treated rats are indicators of hepatocellular damage. This may be due to LPV/r-induced increase in the permeability of cell membrane or liver systol resulting in the release of AST, ALT and ALP into the blood stream (Sarkar et al., 1998). Also, LPV/r could induce oxidative stress or direct liver damage leading to liver

dysfunction and disturbance in the biosynthesis of liver aminotransferases and alkaline phosphatase. In the present study, increases in liver MDA levels with decreases in SOD, GSH and CAT levels obtained in LPV/r treated rats are pointers to oxidative stress through free radical production (Plummer et al., 1981; Fridovich, 1995; Zini et al., 2007). Generally, malondialdehyde is used as an index for lipid peroxidation, and lipid peroxidation is postulated as one of the mechanisms of free radical-induced tissue injury (Lykkesfeldt et al., 2007). Therefore, increases in MDA levels observed in LPV/r treated rats suggest lipid peroxidation. Lipid peroxidation can alter membrane fluidity of liver cells, causing changes in carrier mediated transport, activities of membrane bound enzymes and receptor binding, which could result in the leakage of certain intracellular enzymes (Munyon et al., 1987; Lewis and Zimmerman, 1999).

Interestingly, supplementations with individual doses of MT and ALA prior to treatment with LPV/r decreased serum AST, ALT, ALP and liver MDA levels while liver SOD, CAT and GSH levels were increased. Effects on these parameters were most pronounced in rats supplemented concurrently with MT and ALA. This observed ameliorative effect is consistent with the work of Rishi et al. (2008), which reported the inhibitory effect of MT on endotoxin-induced hepatotoxicity in rats. Similarly, Hussein et al. (2014), reported the protective effects of MT and ALA on cadmium-induced oxidative damage in the liver of rats which is in agreement with our finding. In this study, attenuation of LPV/r-induced hepatotoxicity by MT and ALA pretreatments could be attributed to the inhibition of LPV/r-induced hepatic oxidative stress by these antioxidants (Herrera and Barbas, 2001). In addition, increases in liver SOD, CAT and GSH levels in MT and ALA supplemented animals could be attributed to the stimulatory effects of MT and ALA on the regeneration or the synthesis of these antioxidants. ALA and its reduced form, MT and its metabolites are amphiphilic antioxidants that scavenge free radicals and prevent oxidative stress-induced damage (Pieri et al., 1994; Vriesman et al., 1997; Tan et al., 1998; Trujillo and Radi, 2002). These antioxidants can up-regulate the activities of antioxidants such as SOD, GSH and CAT; thereby, facilitating more antioxidant activities (Bast and Haenen, 2003; Bilska et al., 2008). Due to its small size and high lipophilic nature, MT can cross biological membranes easily and reach all compartments within the cell (Sener et al., 2003), thus protecting DNA, proteins and biological membrane lipids from the deleterious effects of free radicals (Reiter et al., 1993). Moreover, besides its free radical scavenging and antioxidant functions, melatonin's receptor-mediated local functions may contribute to its ability to preserve cell function and limit cell death from apoptosis or necrosis due to oxidative damage (Cabrera et al., 2003; Barrett et al., 2003). Inflammation has been reported as an integral aspect of xenobiotic-induce hepatotoxicity. ALA and MT can inhibit oxidative stress-induced inflammatory cascade characterized by the production of inflammatory mediators (Crespo et al., 80 1999; Kwiecien et al., 2013; Nasole et al., 2014). Furthermore, hepatic damage induced by inflammatory cytokines and other mediators of inflammation was reported to be attenuated by MT (Bellezzo et al., 1998). MT can maintain hepatocytes membrane integrity, thus reducing the leakage of liver enzymes and can inhibit neutrophil infiltration and accumulation in damaged hepatic tissues (Ohta et al., 2000; Sulaiman et al., 2006; Goraca et al., 2011). In the present study, most pronounced effects obtained with combined doses of MT and ALA could be attributed to synergy in antioxidant and anti-inflammatory activities of these antioxidants.

# Conclusion

The present study demonstrated the modulatory effects of MT and ALA on LPV/r-induced hepatotoxicity with most pronounced modulation obtained in rats pretreated concurrently with MT and ALA. Considering the results of this study, MT and ALA could be used for the treatment of lopinavir/ritonavir-associated hepatotoxicity.

## Acknowledgments

The authors would like to appreciate the technical assistance offered by Mr Woy Yirupe of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State

## Conflict of interest

The authors declare no conflict of interest

## Funding sources

The authors declare no sources of funding

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