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Original Article

Melatonin and Alpha Lipoic Acid: Possible Mitigants for Lopinavir/Ritonavir- Induced Renal Toxicity in Male Albino Rats

Elias Adikwu*, Nelson Braimbaifa, Atuboyedia Wolfe Obianime

Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria

Abstract

Introduction: This study evaluated the effects of pretreatments with melatonin (MT), and Alpha Lipoic acid (ALA) on lopinavir/ritonavir (LPV/r) -induced serum levels of creatinine (Cr), urea (U), uric acid (Ua) and kidney levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) in male albino rats. Effects of treatments with MT and ALA were also evaluated on baseline levels of the above parameters.

Materials and Methods: Adult male albino rats were orally administered MT (10mg/kg), ALA (10mg/kg) and LPV/r (22.9/5.71, 45.6/11.4 and 91.4/22.9mg/kg) for 60 days. At the end of drug treatment animals were sacrificed, serum was extracted and evaluated for Cr, U, and Ua. Kidney was harvested and evaluated for MDA, SOD, CAT and GSH.

Results: Treatment with MT and ALA significantly (p<0.05) decreased baseline serum levels of Cr, U, Ua and kidney MDA level while kidney levels of SOD, CAT and GSH were increased when compared to the control. On the contrary, treatment with LPV/r significantly (p<0.05) and dose -dependently increased serum Cr, U, Ua levels and kidney MDA level while kidney levels of SOD, CAT and GSH were decreased when compared to the control. But pretreatments with MT and ALA mitigated LPV/r induced changes in all evaluated parameters. Pronounced mitigation was observed with pretreatment using a combination of MT and ALA.

Conclusion: Observations in this study may be due to the oxidant effect of LPV/r and the antioxidant effects of MT and ALA. This study, therefore recommends MT and ALA as treatment or prevention for LPV/r induced renal toxicity.

Keywords:
Kidney;
Toxicity;
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***Correspondence to:**
E. Adikwu

Tel: +2347068568868

Email:
adikwuelias@gmail.com

Introduction

The kidney is an essential organ in the body for important functions such as maintenance of homeostasis, detoxification and excretion of drugs and their metabolites (Ferguson et al., 2008). Kidney is always exposed to high concentrations of antiretroviral drugs and their metabolites which may lead to accumulation with long term use. Direct or indirect exposure and accumulation of antiretroviral drugs and their metabolites in the kidney could lead to a variety of nephrotoxic effects (Ferguson et al.,

2008).

Lopinavir /ritonavir (LPV/r) combination is an antiretroviral drug used in the management of Human Immunodeficiency Virus (HIV) which has tremendously contributed to decrease in mortality associated with HIV. But therapy with LPV/r might be associated with renal toxicity due to long-term use and possible accumulation in the kidney (Mocroft et al., 2010; Elens et al., 2011; Young et al., 2012). Decrease in creatinine clearance rate, development of kidney stones and chronic kidney diseases were adverse effects reported in patients treated with LPV/r (Orkin et al., 2013; Ryom et al., 2013). Furthermore, interstitial nephritis, increases in creatinine and urea levels have been associated with LPV/r therapy (Chughlay et al., 2005). LPV/r- induced renal toxicity may involve oxidative stress because cumulative data suggest a role for oxidative stress as one of the postulated mechanisms in the pathogenesis of drugs induced renal toxicity (Wolf et al., 1994; Chander et al., 2004; Durak et al., 2004,).

Melatonin and alpha lipoic acid are lipid and aqueous soluble antioxidants that can inhibit oxidative stress via scavenging of oxidative radicals (Tan et al., 2001, Tan et al., 2007). Melatonin and alpha lipoic acid have been reported to prevent lipid peroxidation *in vivo* more efficiently than either vitamins C or E (Reiter et al., 1995). Alpha lipoic acid has a beneficial effect on energy production and is an essential cofactor of mitochondrial complexes (Busse et al., 1992). One of the most beneficial effects of alpha lipoic acid is the ability to regenerate other essential antioxidants such as vitamin C, vitamin E, coenzyme Q10, and glutathione (Packer, et al., 1995; Parker, 1998). In addition to scavenging of reactive oxygen species, melatonin stimulates the antioxidant activities of superoxide dismutase, glutathione peroxidase and catalase (Antolin et al., 1996; Montilla et al., 1997). Interestingly, ALA can inhibit the release of various cytokines, including tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) (Wong et al., 2001). These observations were attributed to the anti-inflammatory and immunomodulatoy effects of ALA (Sola et al., 2005). Also, studies have found that melatonin has anti-inflammatory and immunomodulatoy effects. Because melatonin treatment decreased cell damage, migration of inflammatory cells, reduced serum and tissue inflammatory cytokines levels, tissue lipid peroxidation, and inhibited cell apoptosis in injury mice model (Hu et al., 2009). Studies have shown the beneficial effects of ALA and MT in the prevention or treatment of some pathological conditions characterized by oxidative stress (Abdel-Zaher et al., 2008; Kozirog et al., 2011). This study, therefore evaluated the effects of pretreatments with MT and ALA on LPV/r- induced serum levels of urea, creatinine, uric acid and kidney levels of malondialdehyde, superoxide dismutase, glutathione, and catalase in male albino rats.

Materials and methods

Animals

Eighty five (85) male albino rats of average weight 310 ± 5g used for this study were obtained from the animal house of the University of Port Harcourt, Choba, Rivers State. The animals were housed in a large mesh cage and allowed to acclimatize for 14 days with free access to food and water *adlibitum*.

Drugs

Lopinavir/ritonavir (LPV/r) used for this study was manufactured by Myland Laboratories Limited India. The pure samples of melatonin and alpha lipoic acid used were supplied by Shijiazhuang AO Pharm Import and Export Co Ltd China. All other chemicals used for this study were of analytical grade. LPV/r (22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg) which represent 2, 4 and 8 times the clinical dose of LPV/r, MT (10mg/kg) and ALA (10mg/kg) were used for this study (Hull et al., 2009; Ali, 2013; Bilginoğlu et al., 2014).

Experimental Design

Animals used for this study were divided into 6 groups A-F. Animals in group A served as the control and contained 10 animals which were divided into two groups' A1 and A2. Animals in group A1 (placebo control) were orally treated with normal saline while animals in group A2 (solvent control) were orally treated with 1% ethanol for 60 days.

Animals in group B-F contain 15 animals each which were divided in 3 groups of 5 animals each. Animal in group B were orally treated with 22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg of LPV/r for 60 days. Animals in group C were orally treated with MT (10mg/kg), ALA

(10mg/kg) and a combination of MT and ALA for 60 days. Animals in group D were orally pretreated with 10 mg/kg of MT before oral treatment with 22.9/5.71, 45.6/11.4 and 91.4/22.9mg/kg of LPV/r for 60 days. Animals in group E were orally pretreated with 10mg/kg of ALA before oral treatment with 22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg of LPV/r for 60 days. Animals in group F were orally pretreated with 10mg/kg of MT + 10mg/kg of ALA before oral treatment with 22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg of LPV/r for 60 days.

Collection of Sample for Analysis

Animals were sacrificed using diethyl ether at the end of 60 days of treatment. Blood samples were collected via cardiac puncture. The blood samples were allowed to clot and centrifuged at 1200 rpm for 10 mins using Uniscope centrifuge and serum separated for biochemical analysis. Animals were dissected; kidneys collected and washed in an ice cold 1.15% KCL solution. Kidneys were then homogenized with 0.1M phosphate buffer (pH 7.2). The resulting homogenate was centrifuge at 2500rmp speed for 15 minutes then it was removed from the centrifuge and the supernatant was decanted and stored at -20°C.

Evaluation of Serum Renal Function Parameters

Serum creatinine, urea, and uric acid levels were evaluated using the Clinical Chemistry Autoanalyser RX Series by Randox Laboratories Limited, United Kingdom.

Evaluation of Kidney Oxidative Stress Markers

Kidney malondialdehyde, superoxide dismutase, glutathione and catalase levels were evaluated using the methods reported by Ahmed and Hassainein (2013).

Statistical Analysis

This was done using graph pad prism 5 statistical package and ANOVA for comparison of the means of the various groups. Results are expressed as Mean+ standard error of mean (S.E.M). Statistical significance was set at $p<0.05$.

Results

In this study, treatments with individual doses of MT and ALA for 60 days insignificantly ($p>0.05$) decreased baseline Cr, U, and Ua levels when compared to the control. But significant ($p<0.05$) decreases in the above parameters were obtained when MT and ALA were co-administered when compared to the control (Table 1). Animals that received individual doses of MT and ALA showed significant ($p<0.05$) increases in baseline kidney GSH, CAT and SOD levels with decrease in MDA level when compared to the control. But pronounced and significant ($p<0.05$) increases to 35.7 ± 0.57, 40.7 ± 1.37 and 28.5 ± 0.04 U/mg protein in baseline GSH, CAT and SOD levels with decrease in MDA level to 0.45 ± 0.01 nmole/mg protein were obtained in animals pretreated with combined doses of ALA and MT when compared to the control (Table1).

Table 1: Effects of treatments with melatonin and alpha lipoic acid for 60 days on baseline renal function parameters and kidney oxidative indices in male albino rats

DOSE	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	MDA nmole/mg protein	GSH u/mg protein	CAT u/mg protein	SOD u/mg protein
Control	1.18 ± 0.06	25.8±0.50	1.285±0.09	0.75 ± 0.04	15.3 ± 0.09	17.9 ± 0.02	15.5 ± 0.01
MT	0.90 ± 0.08	20.5±0.85	1.05 ± 0.09	0.67 ± 0.02	25.4±0.69*	26.9 ± 0.65*	24.0 ± 0.33*
ALA	0.9.2±0.02	20.7±0.74	1.10 ± 0.81	0.65 ± 0.78	23.9±0.49*	24.3 ± 1.60*	22.3 ± 0.09*
MT+LA	0.75±0.04*	15.0±0.68*	0.90±0.06*	0.45 ± 0.01*	35.7± 0.57**	40.7±1.37**	28.5 ± 0.04*

MT: Melatonin (10mg/kg) ALA: Alpha lipoic acid (10mg/kg). Results are expressed as Mean ± S.E.M. n=5 * Means significant difference ($p < 0.05$) when compared to the control. ** Means significant difference ($p < 0.05$) when compared to animals treated with individual doses of MT and ALA

Table 2: Effects of pretreatments with melatonin and alpha lipoic acid for 60 days on LPV/r -induced serum levels of creatinine, urea and uric acid in male albino rats

DOSE	LPV/r	MT +LPV/r	ALA+LPV/r	MT+ALA+LPV/r
SERUM CREATININE (Cr) (mg/dl)				
CONTROL	1.18 ± 0.06	1.18 ± 0.06	1.18 ± 0.06	1.18 ± 0.06
22.9/5.71	2.05 ± 0.07	1.12 ± 0.05*	1.17 ± 0.07*	0.931± 0.08*
45.7/11.4	2.59 ± 0.02	1.34 ± 0.09*	1.36 ± 0.03*	0.97± 0.04**
91.4/22.9	3.43 ± 0.08	1.60 ± 0.01*	1.78 ± 0.05*	1.10 ± 0.06**
SERUM UREA (U)(mg/dl)				
CONTROL	25.3 ± 0.50	25.3 ± 0.50	25.3 ± 0.50	25.3 ± 0.50
22.9/5.71	52.8 ± 1.17	25.9 ± 0.49*	27.3 ± 0.25*	21.9± 0.51*
45.6/11.4	69.1 ± 0.51	32.7 ± 0.74*	35.4 ± 1.10*	23.8± 0.68**
91.4/22.9	78.9 ± 0.76	40.6 ± 0.33*	42.4 ± 0.38*	25.7 ± 0.32**
SERUM URIC ACID (Ua) (mg/dl)				
CONTROL	1.27 ± 0.03	1.27 ± 0.03	1.27 ± 0.03	1.27 ± 0.03
22.9/5.71	3.27 ± 0.04	1.29 ± 0.04*	1.30 ± 0.05*	1.20± 0.02*
45.6/11.4	4.85 ± 0.07	1.80 ± 0.02*	1.70 ± 0.07*	1.23± 0.06**
91.4/22.9	5.57 ± 0.05	2.37 ± 0.03*	2.39± 0.01*	1.30 ± 0.04*

MT: Melatonin ALA: Alpha lipoic acid. Results are expressed as Mean ± S.E.M. n=5 * Means significant ($p < 0.05$) difference when compared to LPV/r treated animals. ** Means significant difference ($p < 0.05$) when compared to animals pretreated with individual doses of MT and ALA.

Furthermore, serum levels of Cr, U, and Ua were significantly ($p<0.05$) and dose-dependently increased in animals that received 22.9/5.71-91.4/22.9mg/kg of LPV/r when compared to the control. This study observed maximal increases in serum levels of Cr, U, and Ua to 3.43 ± 0.08, 78.9 ± 0.76 and 5.57 ± 0.05 mg/dl respectively in animals that received 91.4/22.9mg/kg of LPV/r for 60 days. But LPV/r induced increases in these serum values decreased with pretreatment using MT to 1.60 ± 0.01, 40.6 ± 0.33 and 2.37 ± 0.03 mg/dl respectively. Also, pretreatment with ALA decreased these serum values to 1.78 ± 0.05, 42.4 ± 0.38 and 2.39 ± 0.01 mg/dl respectively. These decreases were significantly ($p<0.05$) different when compared to LPV/r treated animals. Pronounced decreases in these serum values to 1.10 ± 0.06, 25.7 ± 0.32 and 1.30 ± 0.04 mg/dl respectively were obtained in animals pretreated with a combination of ALA and MT. These decreases were significantly ($p<0.05$) different when compared to what was produced by pretreatments with individual doses of MT and ALA (Table 2).

Furthermore, this study observed significant ($p<0.05$) dose-dependent increases in kidney MDA levels with decreases in SOD, CAT and GSH levels in animals that received 22.8/5.7-91.4/22.9 mg/kg LPV/r for 60 days when compared to the control. Maximal increase in MDA level to 5.10 ± 0.60 nmole/mg protein with decreases in GSH, SOD and CAT levels to 3.48 ± 0.01, 4.58 ± 0.06 and 3.58 ± 0.06 U/mg/protein respectively were observed in animals that received 91.4/22.9 mg/kg of LPV/r for 60 days. But LPV/r induced increase in MDA level decreased to 2.39 ± 0.07 nmol/mg protein while GSH, SOD and CAT levels increased to 7.38 ± 0.02, 8.30 ± 0.03 and 9.80 ± 0.02 U/mg protein respectively in animals pretreated with MT. Also, pretreatment with ALA decreased MDA level to 2.58 ± 0.08 nmol/mg protein with increases in GSH, SOD and CAT levels to 7.65 ± 0.04, 7.30 ± 0.06 and 8.83 ± 0.08 U/mg protein respectively. Interestingly, pretreatment with combined doses of MT and ALA produced pronounced decrease in kidney MDA level with increases in GSH, SOD and CAT levels which were significantly ($p<0.05$) different when compared to effects produced by pretreatments with individual

Table 3: Effects of pretreatments with melatonin and alpha lipoic acid for 60 days on LPV/r-induced kidney levels of malondialdehyde and glutathione in male albino rats

MALONDIALDEHYDE (MDA) (nmole/mg protein)				
DOSE (mg/kg)	**LPV/r**	**MT +LPV/r**	**ALA+LPV/r**	**MT +ALA+LPV/r**
CONTROL	0.75 ± 0.03	0.75 ± 0.03	0.75 ± 0.03	0.75 ± 0.03
22.9/5.71	2.05 ± 0.09	0.80 ± 0.08*	0.99 ± 0.07*	0.63± 0.06*
45.6/11.4	3.83 ± 0.06	1.10± 0.01*	1.25 ± 0.03*	0. 69± 0.03**
91.4/22.9	5.10 ± 0.60	2.39 ± 0.07*	2.58 ± 0.08*	0.73 ± 0.07**
GLUTATHIONE(GSH) (U/mg protein)				
CONTROL	15.3 ± 0.09	15.3± 0.09	15.3 ± 0.09	15.3 ± 0.09
22.9/5.71	6.32 ± 0.06	14.2 ± 0.02*	13.2 ± 0.04*	16.5± 0.03*
45.6/11.4	4.46 ± 0.05	10.9 ± 0.05*	10.7 ± 0.75*	15.9± 0.05**
91.4/22.9	3.48 ± 0.01	7.38 ± 0.02*	7.65 ± 0.04*	13.7 ± 0.15**

MT: Melatonin ALA: Alpha lipoic acid. Results are expressed as Mean ± S.E.M. n=5 * Means significant ($p < 0.05$) difference when compared to LPV/r treated animals. ** Means significant ($p < 0.05$) difference with respect to animals pretreated with individual doses of MT and ALA.

Table 4: Effects of pretreatments with melatonin and alpha lipoic acid for 60 days on LPV/r induced kidney levels of superoxide dismutase and catalase in male albino rats

SUPEROXIDE DISMUTASE(SOD) (U/mg protein)				
DOSE (mg/kg)	**LPV/r**	**MT +LPV/r**	**ALA+LPV/r**	**MT +ALA+LPV/r**
CONTROL	15.6± 0.01	15.6 ± 0.01	15.5 ± 0.01	15.5 ± 0.01
22.9/5.71	8.20 ± 0.02	13. 7± 0.70*	12.9 ± 0.01*	16.5± 0.02*
45.6/11.4	6.82 ± 0.08	10.3 ± 0.15*	9.03 ± 0.08*	15.5± 0.05**
91.2/22.9	4.58 ± 0.06	8.30 ± 0.03*	7.30 ± 0.06*	14.4 ± 0.07**
CATALASE(CAT) (U/mg protein)				
CONTROL	17.9± 0.02	17.9 ± 0.02	17.9± 0.02	17.9± 0.02
22.9/5.71	7.40 ± 0.03	14.3 ± 0.09*	14.2 ± 0.03*	20.7± 0.08*
45.6/11.4	5.83 ± 0.05	12.8 ± 0.01*	11.9 ± 0.08*	18.6± 0.07**
91.4/22.9	3.58 ± 0.06	9.80 ± 0.02*	8.83 ± 0.04*	18.0 ± 0.03**

MT: Melatonin ALA: Alpha lipoic acid. Results are expressed as Mean ± S.E.M. n=5 * Means significant ($p < 0.05$) difference when compared to LPV/r treated animals. ** Means significant ($p < 0.05$) difference when compared to animals pretreated with individual doses of MT and ALA.

doses of ALA and MT (Table 3-4).

Discussion

Therapy with LPV/r may be associated with renal toxicity characterized by oxidative stress. Also, studies have implicated oxidative stress as one of the possible mechanisms of xenobiotic induced renal toxicity (Huang et al., 2002; Chughlay et al., 2005). MT and ALA are antioxidants with ant-inflammatory and immunomodulatory effects. They can scavenge free radicals in aqueous and lipid medium and prevent oxidative stress induced damage (Hussein et al., 2014). This study, therefore evaluated the effects of pretreatments with MT and ALA on LPV/r induced serum levels of urea, creatinine and uric acid which are markers of renal function (Miller et al., 2005). And kidney levels of MDA, SOD, CAT and GSH which are oxidative stress indices (Adaramoye et al., 2012). Also, effects of treatments with MT and ALA on

baseline levels of the above parameters were evaluated. Treatment with MT and ALA decreased baseline serum urea, creatinine, and uric acid levels. Baseline kidney MDA level was decreased while SOD, CAT and GSH levels were increased in animals treated with MT and ALA. This observation is consistent with previous reports (Adewole et al., 2007; Abd El Salam et al., 2011). On the other hand, dose-dependent increases in serum levels of urea, creatinine and uric acid were observed in animals treated with LPV/r. Also, kidney MDA level was increased while SOD, CAT and GSH levels were decreased in LPV/r treated animals. In this study, it was observed that LPV/r induced increases in serum levels of urea, creatinine and uric acid were reversed in animals pretreated with MT and ALA. Also, kidney MDA level was decreased wile SOD, CAT, GSH levels were increased in animals pretreated with MT and ALA. The alterations of the status of these parameters were pronounced in animals pretreated with a combination of MT and ALA which could be attributed to their synergistic effects.

In this study, increases in serum urea, creatinine and uric acid levels observed in LPV/r treated animals are indicators of kidney damage. This observation is consistent with previous reports (Lockman et al., 2012). Observed increases in serum urea, creatinine and uric acid levels in LPV/r treated animals may be due to LPV/r induced oxidative stress in the kidney of treated animals. Because oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; and thus reducing glomerular filtration rate (Rezzani et al., 2005; Hagar et al., 2006). In this study, dose-dependent increases in kidney MDA levels with decreases in SOD, CAT, GSH levels observed in LPV/r treated animals are indicators of lipid peroxidation and oxidative stress (Adewole et al., 2007; Adaramoye et al., 2012). Oxidative stress can cause kidney mitochondria damage which may stimulate reactive oxygen species production leading to the depletion of kidney antioxidants (Galle, 2001). Lipid peroxidation can stimulate the production of reactive electrophiles, such as epoxides and aldehydes that are capable of modifying kidney DNA, protein, and other macromolecules. Lipid peroxidation can impair normal kidney function by increasing membrane permeability, inactivating membrane-bound receptors or enzymes, and promoting efflux of cytosolic solutes (Avery, 2011, Deavall et al., 2012). Reports have shown that antiviral agents induced kidney injury can occur through three major pathways. (1) The over-expression or competitive inhibition of transport pumps like the hOAT family or multidrug resistance-associated protein 2 (MRP 2) or 4 (MRP 2,4) which can lead to tubular cell toxicity. (2) The stimulation of mitogen-activated protein kinase pathway which can affect barrier function in renal epithelial cell cascade. (3) Through oxidative stress by the production of reactive oxygen species which can damage mitochondria, disrupting fatty-acid oxidation, and energy production (Ho et al., 2000; Cihlar et al., 2002).

Furthermore, observed decreases in serum levels of creatinine, urea and uric acid in MT and ALA pretreated animals may be due to the direct effects that these antioxidants exert on kidney metabolism and urine production (Richardson et al. 1992). Inhibition of oxidative stress induced production of vasoactive mediators thereby preventing renal vasoconstriction may contribute to the renal protective effects of MT and ALA observed in this study (Paulis and Simko, 2007, Reiter et al. 2009). Another mechanism through which MT and ALA exhibited protective effects could be the inhibition of mitogen activated protein kinase pathway which has been reported in some studies (Mao et al., 2010; Sun et al., 2015). Observed increases in kidney GSH, CAT and SOD in animals pretreated with MT and ALA may be due to the active stimulation or synthesis of these antioxidant enzymes (Leon et al., 2004). Melatonin is known to increase tissue mRNA levels of SOD (Antolin et al., 1996) and also, stimulates CAT activity (Kim et al., 2000). ALA has been reported to increase expression of superoxide dismutase gene which is a factor for the production of SOD (Nistico et al. 1992). Studies have shown that ALA contains thiol groups which are essential precursors or intermediates used in GSH synthesis (Packer et al., 1995; Lilling and Holmgren, 2007). ALA can facilitate transport of cystine, into cells where it is converted to cysteine for the biosynthesis of GSH (Packer, 1998). ALA has been previously reported to mediate induction of GSH through transcription factor Nrf2 thus maintaining GSH availability for combating oxi-dative stress (Kilic et al., 1998; Moini et al., 2002).

Furthermore, MT and ALA are lipid soluble agents; capable of penetrating the aqueous environments of cells. This amphiphilic nature allows these agents to protect membranes, cytosolic molecules and nuclear DNA from free radical damage and also stabilize membrane (Reiter et al., 1991; 1995; Kagan_et al., 1992). These antioxidants are known to have anti-inflammatory properties and therefore could prevent kidney damage induced by inflammation (Kang et al., 2009; Shokrzadeh et al., 2014).

Conclusion

This study demonstrated that pretreatments with individual doses of MT and ALA mitigated LPV/r induced changes in renal function parameters and kidney oxidative stress markers. Pronounced mitigation was observed with pretreatment using combined doses of MT and ALA which may be due to their synergistic effects. This study, therefore recommends MT and ALA as prevention or treatment for LPV/r induced renal toxicity.

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Conflict of Interest

The authors declare no conflict of interest.

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