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Melatonin and alpha lipoic acid restore electrolytes and kidney morphology of lopinavir/ritonavir-treated rats

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ABSTRACT

Introduction: Lopinavir/ritonavir (LPV/r) may cause renal dysfunction such as electrolyte and acid base disorders and alteration in kidney morphology. Drug-induced renal dysfunction can occur through multiple mechanisms including oxidative stress and inflammation.**Objectives:** The current study aimed at evaluating the protective effects of melatonin (MT) and alpha lipoic acid (ALA) against serum electrolytes and kidney histology of LPV/r-treated rats. Adult albino rats were randomized into six groups (A to F). Rats in the control groups were treated orally with normal saline and 1% ethanol as placebo and solvent control for 90 days respectively. Rats in the experimental groups were pre-treated orally with 10 mg/kg of MT, 10 mg/kg of ALA, and MT+ ALA daily before treatment with 22.9/5.71, 45.6/11.4 94 and 91.4/22.9 mg/kg/d of LPV/r for 90 days respectively.**Materials and Methods:** At the end of treatment, rats were euthanized. Blood samples were collected and serum samples were extracted and evaluated for electrolytes, total protein, and albumin. Additionally, kidneys were excised via dissection and evaluated for morphological changes.**Results:** Significant ($P < 0.001$) decreases in serum sodium, potassium, chloride, bicarbonate, total protein and albumin in a dose-dependent fashion were obtained in LPV/r-treated rats when compared to control. Dose-dependent kidney morphological changes characterised by tubular necroses were obtained in LPV/r-treated rats. The observations in LPV/r-treated rats were significantly reversed in MT ($P < 0.01$), ALA ($P < 0.01$) and MT+ALA ($P < 0.001$) pre-treated rats when compared to LPV/r-treated rats.**Conclusion:** MT and ALA can serve as adjuvant therapies for LPV/r-associated alterations in serum electrolytes and kidney histology.**Implication for health policy/practice/research/medical education:**

This study discovered that melatonin and alpha lipoic acid may have clinical applications as adjunct therapies for lopinavir/ritonavir associated alterations in serum electrolytes and kidney morphology.

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Introduction

The use of lopinavir/ritonavir (LPV/r) as an essential component of highly active antiretroviral therapy (HAART) has improved the prognosis of human immunodeficiency virus (HIV)-infected people. It has reduced the risk of early death from opportunistic infections and other consequences that could arise as a result of HIV infection. Nonetheless, LPV/r use could be characterized by some adverse consequences like renal dysfunction which may have higher risk than its therapeutic benefit (1). Clinically, HAART can precipitate various forms of renal dysfunctions including electrolyte and acid base disorders, acute kidney injury, lactic

acidosis, and chronic kidney disease (2). Additionally, acute tubular necrosis, interstitial infiltrates, fibrosis, and edema, with cytopathic changes involving proximal and distal tubules are some evident features of HAART associated renal pathology (3). The aforementioned kidney injuries can occur through multiple mechanisms, including oxidative stress, inflammation, direct tubular toxicity, and allergic reactions (4). Oxidative stress through free radical production leading to lipid, DNA, and protein damage with the activation of apoptotic pathways, and the initiation of inflammatory response has been implicated in drug-induced kidney injury (5).

Melatonin (MT) is a natural neurotransmitter-like

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compound produced by the pineal gland. It is involved in the biologic and physiologic regulations of body functions. MT is an important antioxidant and also a wide spectrum anti-inflammatory molecule, that modulates both pro- and anti-inflammatory cytokines in different pathophysiological conditions (6). It acts as a universal, multifunctional and chain breaking antioxidant through direct free radical scavenging, and the stimulation of anti-oxidative enzymes. It also increases the effectiveness of mitochondrial oxidative phosphorylation and diminishes electron leakage (thus reducing free radical generation) (7). MT and its metabolites can produce synergistic reaction cascade against reactive oxygen species (ROS) and reactive nitrogen species (RNS). This amplifies its efficacy and expands its spectrum of action against oxidative and nitrosative stress (8). Its high lipophilicity and hydrophilicity enables it to offer both intracellular and extracellular antioxidant activity in cells (9). In addition, it has shown potential health benefits in diseases such as cancer, hypertension, diabetes and obesity (9).

Alpha-lipoic acid (ALA), also known as thioctic acid is an essential cofactor in mitochondrial dehydrogenase reactions. It is soluble in water and lipid, and is widely distributed in cellular membrane, cytosol, and extracellular space (10). It acts as a redox regulator of thiol-containing proteins with anti-inflammatory and immunomodulatory activities and has potential for the treatment of some disease conditions (11). The anti-inflammatory effect of ALA includes inhibition of pro-inflammatory cytokines and the stimulation of anti-inflammatory cytokines (12). The redox activity of ALA and its reduced form dihydrolipoic acid (DHLA) is characterized by direct scavenging of free radicals, both *in-vitro* and *in-vivo* and the regeneration of other essential antioxidant molecules such as coenzyme Q10, vitamin C and vitamin E. In addition, ALA/ DHLA can prevent proteins, lipids, and DNA from damage caused by oxidative stress (13).

Objectives

The current study aimed at evaluating the protective effects of MT and ALA against a rat model of LPV/r-induced alterations in serum electrolytes and kidney histology.

Materials and Methods

Animal care

Eighty-five adult albino rats were sourced from the animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria. The albino rats were adapted to laboratory conditions one week prior to the beginning of the experiment. The albino rats were maintained at standard temperature (22-24°C) and a period of 12 hr light, and 12 hr darkness. The albino rats had free access to food and water *ad libitum*. The albino rats were randomised into six groups A to F. Group A (Control) contained 10 albino rats which were sub-divided

into two groups' A1 (placebo control) and A2 (solvent control) of 5 albino rats each. Groups B-F contained 15 albino rats each which were sub-divided into three groups of 5 albino rats each.

Drugs and chemicals

MT and ALA were supplied by AO Pharm Import and Export Co Ltd China whereas LPV/r was manufactured by Myland Laboratories Limited India. The doses of LPV/r which represent 2, 4 and 8 times the clinical dose (14), 10 mg/kg of MT and 10 mg/kg of ALA were used for this study (15, 16). Water was used as the solvent for ALA (17), whereas 1% ethanol was used as the solvent for LPV/r (18). MT was dissolved in 1% ethanol and diluted with normal saline (19).

Drug administration

Rats in group A1 (placebo control) and group A2 (solvent control) were treated orally with 0.2 mL of normal saline and 1% ethanol for 90 days respectively. Rats in group B were treated orally with 22.9/5.71, 45.6/11.4 and 91.4/22.9 mg/kg/d of LPV/r for 90 days respectively. Rats in group C were treated orally with 10 mg/kg of MT, 10 mg/kg of ALA, and MT +ALA daily for 90 days respectively. Rats in groups D- F were pre-treated orally with 10 mg/kg of MT, 10 mg/kg of ALA, and MT+ALA daily prior to treatment with 22.9/5.71, 3 45.6/11.4 and 91.4/22.9 mg/kg/d of LPV/r for 90 days respectively.

Animal sacrifice, sample collection and evaluation

At the end of treatment, albino rats were fasted overnight and sacrificed with the aid of diethyl ether and blood samples were collected in plain bottles. The blood samples were allowed to clot and retract after which serum samples were isolated after centrifugation at 2000 g for 15 minutes. Serum samples were analysed for potassium (K⁺), sodium (Na⁺), bicarbonate (HCO₃⁻), and chloride (Cl⁻), albumin and total protein using standard laboratory reagents. The albino rats were dissected kidney tissues were collected, cleaned, processed using standard laboratory technique and stained with hematoxylin and eosin (H & E). Prepared kidney tissues were examined for pathology with the aid of a microscope.

Statistical analysis

Differences between groups were compared using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test using. Data was statistically analyzed using SPSS/17.0 software. Differences were considered significant at <0.01 and <0.001.

Ethical issues

The research was approved by the Research Ethics Committee of the University of Port Harcourt, Nigeria. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics

Table 1. Effects of melatonin and alpha lipoic acid on serum electrolytes, total protein and albumin of albino rats

Group	K ⁺ (mmol/L)	Na ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ₃ ⁻ (mmol/L)	Total protein (g/dL)	Albumin (g/dL)
Control (Placebo)	2.66±0.56	110.1±9.63	106.1±8.66	10.9±0.33	4.50±0.71	2.88±0.23
MT	2.75±0.27	119.7±7.43	108.6±8.70	11.2±0.25	4.65±0.26	2.90±0.25
ALA	2.70±0.38	116.0±9.57	106.9±9.50	11.0±0.77	4.60±0.32	2.92±0.17
MT+ALA	2.80±0.76	128.7±8.16	119.4±7.95	11.6±0.32	4.69±0.57	2.97±0.10

Data expressed as Mean ± SEM, n=5, MT; melatonin, ALA; alpha lipoic acid, K; potassium, HCO₃⁻; bicarbonate.

Committee of University of Port Harcourt, Nigeria.

Results

Serum Na⁺, K⁺, Cl⁻ and HCO₃⁻ levels were normal ($P > 0.05$) in rats treated with MT, ALA and MT+ALA when compared to control (Table 1). On the other hand, serum Na⁺, K⁺, Cl⁻ and HCO₃⁻ were significantly ($P < 0.001$) decreased in a dose-dependent fashion in rats treated with LPV/r when compared to control (Tables 2 and 3). However, the serum levels of the aforementioned parameters were significantly restored in rats pre-treated with MT ($P < 0.01$) and ALA ($P < 0.01$) when compared to LPV/r-treated rats. Interestingly pre-treatment with MT+ALA significantly restored the serum levels of Na⁺, K⁺, Cl⁻ and HCO₃⁻ at $P < 0.001$ when compared to LPV/r-treated rats (Tables 2 and 3). The serum levels of

total protein and albumin were normal ($P > 0.05$) in rats treated with MT, ALA and MT+ALA when compared to control (Table 1). However, in a dose-dependent fashion, significant ($P < 0.001$) low serum levels of total protein and albumin were obtained in rats treated with LPV/r when compared to control (Table 4). On the other hand, serum total protein and albumin levels were significantly restored in MT ($P < 0.01$), ALA ($P < 0.01$) and MT+ALA ($P < 0.001$) pre-treated rats when compared to LPV/r-treated rats (Table 4). Furthermore, the examined sections of the kidneys of rats treated with MT, ALA and MT+ALA showed normal histology when compared to control (Figures A to D). However, the kidneys of rats treated with LPV/r showed pathology characterised by tubular necroses (Figure E). The kidneys of rats pre-treated with individual doses of MT and ALA showed tubular necroses

Table 2. Effects of melatonin and alpha lipoic acid on serum potassium and bicarbonate of lopinavir/ritonavir-treated albino rats

Dose (mg/kg)	Control	LPV/r	MT	ALA	MT + ALA
Serum K⁺ (mmol/L)					
22.8/5.71	2.66±0.56	2.00±0.63*	2.50±0.53**	2.47±0.44**	2.61±0.44**
45.6/11.4	2.66±0.56	1.55±0.57*	2.07±0.91**	2.00±0.67**	2.58±0.67***
91.2/22.9	2.66±0.56	1.00±0.06*	1.56±0.24**	1.40±0.09**	2.40±0.11***
Serum HCO₃⁻ (mmol/L)					
22.8/5.71	10.9±0.33	5.53±0.60*	7.67±0.33**	7.60±0.25**	10.0±0.57***
45.6/11.4	10.9±0.33	3.07±0.45*	5.50±0.51**	5.31±0.11**	8.94±0.23***
91.2/22.9	10.9±0.33	2.05±0.53*	3.90±0.22**	3.85±0.31**	7.83±0.17***

Data expressed as Mean ± SEM, n=5, MT=melatonin, ALA; alpha lipoic Acid, K; potassium, HCO₃⁻; bicarbonate, Cl; chloride, Na; sodium.

* Significant ($P < 0.001$) difference when compared to control.

** Significant ($P < 0.01$) difference when compared to LPV/r.

*** Significant ($P < 0.001$) difference when compared to LPV/r.

Table 3. Effects of melatonin and alpha lipoic acid on serum chloride and sodium of lopinavir/ritonavir-treated albino rats

Dose (mg/kg)	Control	LPV/r	MT	ALA	MT + ALA
Serum Cl⁻ (mmol/L)					
22.8/5.71	106.1±8.66	60.5±5.03	88.8±6.67**	80.5±6.53**	100.4±8.41**
45.6/11.4	106.1±8.66	51.0±4.57	73.0±5.71**	70.7±4.32**	95.2±6.65***
91.2/22.9	106.1±8.66	40.2±2.16	61.6±3.62**	58.1±3.47**	91.1±7.47***
Serum Na⁺ (mmol/L)					
22.8/5.71	110.5±9.63	63.3±6.63*	83.6±6.27**	82.3±6.31**	100.1±9.10**
45.6/11.4	110.5±9.63	44.2±3.57*	68.9±7.13**	65.9±4.51**	97.4±7.32***
91.2/22.9	110.5±9.63	36.0±2.16*	63.0±6.27**	59.4±3.71**	90.0±8.57***

Data expressed as mean ± SEM, n=5, MT; melatonin, ALA; alpha lipoic acid, Cl; chloride, Na; sodium.

* Significant ($P < 0.001$) difference when compared to control.

** Significant ($P < 0.01$) difference when compared to LPV/r.

*** Significant ($P < 0.001$) difference when compared to LPV/r.

Table 4. Effects of melatonin and alpha lipoic acid on serum total protein and albumin of lopinavir/ritonavir-treated albino rats

Dose (mg/kg)	Control	LPV/r	MT	ALA	MT + ALA
Serum Total Protein (g/dL)					
22.8/5.71	4.50±0.71	2.00±0.44*	3.33±0.33**	3.21±0.20**	4.22±0.63***
45.6/11.4	4.50±0.71	1.52±0.01*	2.07±0.51**	2.00±0.32**	4.10±0.41***
91.2/22.9	4.50±0.71	1.17±0.08*	1.75±0.22**	1.60±0.01**	4.00±0.32***
Serum Albumin (g/dL)					
22.8/5.71	2.88±0.23	2.10±0.63*	2.66±0.53**	2.40±0.32**	2.70±0.34**
45.6/11.4	2.88±0.23	1.62±0.07*	2.09±0.91**	2.00±0.75**	2.66±0.24***
91.2/22.9	2.88±0.23	1.00±0.06*	1.52±0.04**	1.41±0.03**	2.57±0.55***

Data expressed as mean ± SEM, n=5, MT; melatonin, ALA; alpha lipoic acid.

* Significant (p<0.001) difference when compared to control.

** Significant (p<0.01) difference when compared to LPV/r.

*** Significant (p<0.001) difference when compared to LPV/r.

respectively (Figures F and G). However, the kidney of rat pre-treated with MT+ ALA showed normal histology (Figure H).

Discussion

Kidney plays a major role in the metabolism and excretion of waste products of metabolism including drug metabolites (20). However, the physiological functions of the kidney can be impaired due to its vulnerability to various forms of toxic insults (21). Kidney injury ranging from electrolyte dysfunction to evident pathology in the structure of kidney can arise from the use of LPV/r (22). Renal pathology associated with xenobiotics can occur through different mechanisms including oxidative stress and inflammation. MT and ALA have antioxidant and anti-inflammatory effects therefore, the current study examined the protective effects of MT and ALA against a rat model of LPV/r-induced alterations in serum electrolytes and kidney histology. Electrolytes which include Na⁺, K⁺, Cl⁻ and HCO₃⁻ are charged low molecular mass molecules present in plasma and cytosol. Electrolytes have essential functions which include water balance, maintenance of pH, regulation of heart and muscle function, electron transfer reactions as well as serving as cofactors for enzymes. Specifically, Na⁺ is the major extracellular cation that maintains the normal distribution of water and osmotic pressure in various fluid compartments (23). Serum K⁺ is the major intracellular cation that regulates heartbeat and the functions of the muscles in addition to other functions (23). On the other hand, Cl⁻ in conjunction with Na⁺ are involved in the maintenance of water distribution, osmotic pressure and anion-cation balance in extracellular fluid. Studies have shown that the evaluations of body fluid concentrations of Na⁺, K⁺, Cl⁻ and HCO₃⁻ are clinically used as electrolyte profile in the assessment of kidney disorder (24). In the current study, normal serum Na⁺, K⁺, Cl⁻ and HCO₃⁻ levels were observed in rats treated with MT, ALA and MT+ALA. On the other hand, the above serum electrolytes were decreased in a dose-dependent fashion in rats treated with LPV/r.

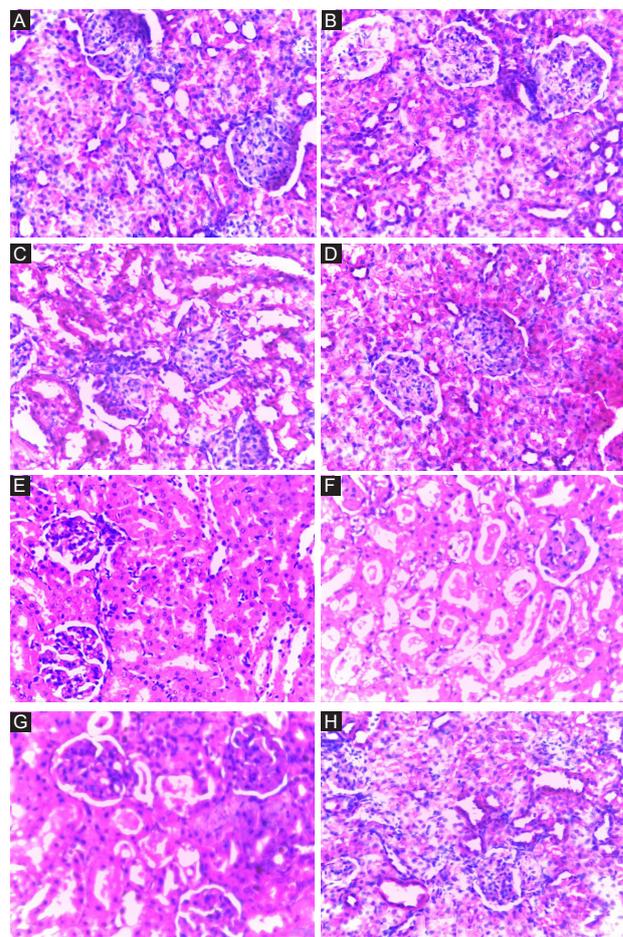


Figure 1. A: Kidney of control rat showing normal tubules. B: Kidney of rat treated with 10 mg/kg/d of melatonin (MT) showing normal tubules. C: Kidney of rat treated with 10 mg/kg/d of alpha lipoic acid (ALA) showing normal tubules. D: Kidney of rat treated with a combination of MT and ALA showing normal tubules. E: Kidney of rat treated with 45.6/11.4 mg/kg/d of lopinavir/ritonavir showing tubular necrosis. F: Kidney of rat treated with 10 mg/kg/d of MT and 45.6/11.4 mg/kg/d of lopinavir/ritonavir showing tubular necrosis. H: Kidney of rat treated with 10 mg/kg/d of ALA and 45.6/11.4 mg/kg/d of lopinavir/ritonavir showing tubular necrosis. G: Kidney of rat treated with 10 mg/kg/d of ALA and 10 mg/kg/d of MT and 45.6/11.4 mg/kg/d of lopinavir/ritonavir showing normal tubules (H&E) × 400.

However, the serum levels of Na^+ , K^+ , Cl^- and HCO_3^- were restored in rats pre-treated with individual doses of MT and ALA. Interestingly, most restored levels of Na^+ , K^+ , Cl^- and HCO_3^- occurred in MT+ALA pre-treated rats. Studies have shown that increased renal disposal of protein often characterise chronic kidney disease and is an established predictor of chronic kidney disease progression that reflects both glomerular and tubulointerstitial injury (25). This study observed normal serum levels of total protein and albumin in rats treated with MT, ALA and MT+ ALA. However, in a dose-dependent fashion, serum levels of total protein and albumin were decreased in rats treated with LPV/r. This observation is a sign of renal dysfunction that has been previously reported (26). On the other hand, the serum levels of the aforementioned parameters were restored in rats pre-treated with individual dose of MT, and ALA with most restored levels observed in MT+ ALA pre-treated rats. Furthermore, the examined sections of the kidneys of rats treated with MT, ALA and MT+ ALA showed normal histology. In contrast, the kidneys of rats treated with LPV/r showed pathology characterised by tubular necroses. The observation in LPV/r-treated rats is in agreement with earlier reports (27). Interestingly, this study observed decreased tubular necroses in the kidneys of rats pre-treated with individual doses of MT and ALA. On the other hand, tubular necrosis was completely abrogated in rat pre-treated with MT+ALA.

Therapy with LPV/r can produce electrolytes and acid-base derangements with progressive loss of kidney function. Electrolytes and acid-base alterations form a major part of the pathological disease processes associated with renal failure (28). In the current study, the mechanisms by which LPV/r caused alterations in serum electrolytes and kidney histology are not well understood. However, studies have shown that drug-induced renal dysfunction could be associated with the following mechanisms; altered intra-glomerular hemodynamics, tubular cell toxicity, oxidative stress, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy (29). Recently, the place of oxidative stress in drug-induced renal dysfunction has gained so much attention. This has been attributed to drug or metabolite-induced free radical production which can stimulate biomolecular damage in the kidney through the initiation of LPO, inflammation and the stimulation of apoptotic pathways (30). Oxidative stress could be one of the primary mechanisms by which LPV/r causes renal dysfunction because it has been associated with free radical generation and the perturbation of kidney antioxidant defense (31). The protective effects of MT and ALA observed in this study may be due to their inhibitory effects on the oxidative and inflammatory actions of LPV/r in the kidneys of treated rats. MT is a multifunctional, universal and chain breaking antioxidant that forms reaction cascade against oxidants. Furthermore, it regulates a number of inflammatory

reactions by up-regulating anti-inflammatory and inhibiting pro-inflammatory cytokines (32). ALA is a thiol containing antioxidant that terminates oxidative and nitrosative stress by scavenging and neutralizing ROS and RNS. Besides, it is an anti-inflammatory agent that inhibits processes associated with the production of mediators of inflammation *in-vitro* and *in-vivo* (33). In this study, the nephroprotective effect observed with the concurrent use of MT and ALA could be attributed to their stimulatory effects on each other.

Conclusion

MT and ALA can serve as adjuvant therapies for LPV/r associated alterations in serum electrolytes and kidney morphology.

Authors' contribution

EA; Study design, literature search, animal handling and treatment, data collection, data analysis, manuscript preparation and revision. BN; Study design, literature search, literature review, manuscript editing, manuscript preparation and revision. WAO; Study design, literature search, literature review, manuscript editing, manuscript preparation and revision.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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