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Research Article

Effect of co-administered lopinavir/ritonavir and sulfamethoxazole/trimethoprim on kidney function and architecture of albino rats

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ABSTRACT

Background: Human immunodeficiency virus/acquired immune deficiency syndrome is usually associated with co-infections which has allow the concurrent use of sulfamethoxazole/trimethoprim (SMX/TMP)+Lopinavir/ritonavir (LPV/r). The concurrent use of these drugs may have possible adverse effects on the kidney because they are individually associated with adverse renal events. This study, therefore evaluates the possible effect of co-administered SMX/TMP+LPV/r on kidney function and architecture of albino rats.

Methods: Seventy five (75) animals which were divided into five groups were used in this study. Group A, which served as control, contained 15 animals which were treated with 1% ethanol orally. Group B-E, which contained 15 animals each, was further subdivided into three groups of five animals each. Animals in these groups were treated with oral doses of SMX/TMP (11.2/2.3 mg/kg), LPV/r (11.4/2.9 mg/kg), and combined doses of SMX/TMP+LPV/r for 2-8 weeks respectively. Serum levels of creatinine, urea, and uric acid were evaluated. Kidney tissue was evaluated for malondialdehyde (MDA), superoxide dismutase (SOD), and histopathological changes.

Results: Treatment with single doses of SMX/TMP, and LPV/r, produced a time-dependent increase in serum creatinine and urea. But no significant synergistic effects on serum creatinine and urea were observed when these agents were co-administered. Treatment with single and combine doses of these agents had no significant effects on serum uric acid level. Treatment with single agents produced time-dependent increase in kidney MDA and decrease in SOD level but without any significant effects when these agents were co-administered. Kidney of animals treated with single doses of these agents showed hypercellarity of the interstitium of the glomeruli and vascular congestion with no synergistic effects when these agents were co-administered.

Conclusion: Concurrent use of SMX/TMP+LPV/r in the management of HIV and co-infection may not have any deleterious effect on the renal system.

Keywords: Kidney, Lopinavir/Ritonavir, Sulfamethoxazole/Trimethoprim, Toxicity, Rats

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) is the leading cause of death in sub-Sahara Africa. In 2007, the African region contained an estimated 68% of all people living with AIDS and recorded 76% of all AIDS deaths, with 1.7 million new infections. This brings the number of people living with HIV to 22.5 million, and with 11.4 million AIDS orphans living in the region.[1,2] Similar to the situation in most countries in Sub-Saharan region, AIDS continued to be the leading cause of mortality in other countries of the world.[3] But the introduction and availability of highly active antiretroviral therapy has dramatically improved the prognosis of HIV-infected persons to a level close to a normal life expectancy and has significantly reduced HIV-associated mortality.[4] Studies have shown that morbidity and mortality reported in HIV patients are mainly related to co-morbidities,

co-infections, and other health related conditions. The high rate of co-morbidities and co-infections in people with HIV is having an extremely negative effect on their health and well-being. They also have serious implications for health care costs as many of these conditions result in hospitalization and extended use of health care services.[5] Irrespective of the increase in the health bill, co-morbidities, and co-infections increase pill burden due to the concurrent use of antiretroviral drugs with other drugs which may be detrimental to tissues and organs of the body including the kidney which is the primary organ of drug excretion.

Due to co-infections associated with HIV/AIDS, sulfamethoxazole/trimethoprim (SMX/TMP) was recommended as prophylaxis for people living with HIV/AIDS in Africa with symptomatic HIV diseases (WHO Stage 2.3 or 4) and asymptomatic individuals who have a CD4 count of ≤500 cells/mm.[3,6] Due to better outcome with the use of SMX/TMP, it has been used in combination with antiretroviral drugs in cases of co-infections. One of such cases is the concurrent use of SMX/TMP with lopinavir/ritonavir (LPV/r), containing antiretroviral regimens in patients with HIV and *Pneumocystic jurevici pneumonia*.[7] The concurrent use of this combination may have deleterious effect on the renal system since these drugs are individually implicated in adverse renal events.

Kidney is the major organ of excretion and homeostasis for water-soluble molecules; it can concentrate certain substances actively. It is responsible for bioconversion of chemicals and metabolically activates a variety of chemical substances.[8] Drugs and chemical substances are transported across the renal proximal tubule by transporters and excreted via the urine. Drugs and chemical substances may become toxic when in contact with drug transporters in tubular cells which may lead to nephrotoxicity and subsequently renal failure.[9] Co-administration of drugs may increase functional burdens on the kidney via the process of drug excretion. This may possibly lead to adverse events that could alter the function and architecture of the kidney; hence, this study evaluates the possible effect of the co-administration of LPV/r and SMX/TMP on kidney function and architecture of albino rats.

METHODS

Drugs

Drugs used in this work are LPV/r manufactured by Myland Laboratories Limited India, and SMX/TMP manufactured by CSPC Ouyi Pharmaceuticals LTD No. 276 Zhongshen West Road Shijiazhuang, China. Both drugs were of analytical grade.

Animals

The animals used in this research work were obtained from the Animal House of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The animals were allowed free access to food and water *ad libitum* and were allowed to acclimatize for 14 days. Animals were handled according to Helsinki declaration on the handling and use of animals.

Dose selection

11.2/2.3 mg/kg of SMX/TMP and 11.4/2.9 mg/kg of LPV/r were used in this study. Doses used are within the clinically recommended dose range.[10,11]

Preparation of drug

LPV/r tablets were crushed and dissolved in 1% ethanol while SMX/TMP tablets were also crushed and suspended in water.[12]

Grouping of animals

65 healthy male rats were weighed and housed in a large mesh cage. The animals were divided into five Groups A B C D and E.

Drug administration

Group A: This served as the control and contained fifteen animals which were treated with 1% ethanol orally throughout the duration of the study.

Group B: This group contained 15 animals which were further divided into three subgroups (B1-B3). Animals in subgroup B1 were treated with 11.2/2.3 mg/kg of SMX/TMP. Animals in subgroup B2 were treated with 11.4/2.9 mg/kg of LPV/r. Animals in subgroup B3 were treated with combined doses of SMX/TMP+LPV/r. All animals in this group were treated for 2 weeks.

Group C: This group contained 15 animals which were further divided into four subgroups (C1-C3). Animals in subgroup C1 were treated with 11.2/2.3 mg/kg of SMX/TMP. Animals in subgroup C2 were treated with 11.4/2.9 mg/kg of LPV/r. Animals in subgroup C3 were treated with combined doses of SMX/TMP+LPV/r. All animals in this group were treated for 4 weeks

Group D: This group contained 15 rats which were further divided into four subgroups (D1-D3). Animals in subgroup D1 were treated with 11.2/2.3 mg/kg of SMX/TMP. Animals in subgroup D2 were treated with 11.4/2.9 mg/kg of LPV/r. Animals in subgroup D3 were treated with combined doses of SMX/TMP+LPV/r. All animals in this group were treated for 6 weeks.

Group E: This group contained 15 animals which were further divided into four subgroups (E1-E3). Animals in subgroup E1 were treated with

11.2/2.3 mg/kg of SMX/TMP. Animals in subgroup E2 were treated with 11.4/2.9 mg/kg of LPV/r. Animals in subgroup E3 were treated with combined doses of SMX/TMP+LPV/r. All animals in this group were treated for 8 weeks.

Collection of sample for analysis

Animals were sacrificed using chloroform anesthesia at the end of 2, 4, 6, and 8 weeks of treatment, respectively. Blood sample was collected from the common carotid artery. The sample was allowed to clot and centrifuged at 1000 rpm for 5 mins using Uniscope centrifuge and serum separated for analysis. Rats were dissected kidney was collected, weighed, and analyzed for histopathological changes.

Preparation of tissue homogenate

The kidney tissues were homogenized using 0.1% triton X-100 buffer (pH 7.4). The homogenate was centrifuged at 12,000 rpm and 4°C for 30 mins, and the supernatant was used as a sample for biochemical investigations.[13]

Biochemical estimations

Malondialdehyde (MDA) and superoxide dismutase (SOD)

The liver MDA concentrations, a lipid peroxidation index, were determined spectrophotometrically according to Draper and Hadley, 1990.[14] SOD activities were estimated according to Beauchamp and Fridovich.[15]

Serum urea, uric acid and creatinine

Serum urea, uric acid, and creatinine levels were determined using photoelectric colorimeter as described by.[16-18]

Histopathological analysis

For light microscopic examination, kidney tissues from each group were fixed with 10% buffered formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 cm in thickness were prepared and stained with hematoxylin and eosin[19] and then examined under light microscopy. The photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

Statistical analysis

Results were expressed as mean±S.E.M. Statistical analysis was done with the aid of SPSS for windows; SPSS Inc., Chicago, Standard version 14.0 to determine the difference between mean using one-way analysis of variance.

RESULTS

Effects on serum urea, uric acid, creatinine and kidney weight

The effects of SMX/TMP, LPV/r, and SMX/TMP+LPV/r on kidney weight, serum creatinine urea, and uric acid are shown in Tables 1-4. This result shows that treatment with single and combine doses of these agents for 2-8 weeks did not produce any significant ($p > 0.05$) change in kidney weight with respect to the control (Table 1).

Treatment with SMX/TMP produced a time-dependent increase in serum creatinine which is significant ($p < 0.05$) in week 6 and 8 with respect to control (Table 2). Animals exposed to LPV/r produced significant ($p < 0.05$) increase in serum creatinine in week 8 when compared with the control. Furthermore, time dependent increase in serum creatinine which was significant ($p < 0.05$) in week 6

Table 1: Effects of LPV/r, SMX/TMP, and their combination on kidney weight (g) in rats.

Dose	Week 2	Week 4	Week 6	Week 8
Control	0.78±1.35	0.76±2.14	0.73±3.07	0.77±2.31
SMX/TMP (11.2/2.3 mg/kg)	0.75±1.08	0.72±3.07	0.79±0.11	0.79±2.03
LPV/r (11.4/2.9 mg/kg)	0.76±5.03	0.75±0.24	0.75±3.18	0.74±0.01
SMX/TMP+LPV/r	0.76±6.03	0.8±0.05	0.77±5.08	0.76±1.04

Results are express as mean±SEM, n=5, SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/ritonavir

Table 2: Effects of LPV/r, SMX/TMP, and their combination on serum creatinine (µmol/L) in rats.

Dose	Week 2	Week 4	Week 6	Week 8
Control	105.75±3.17	102.64±2.04	104.55±2.15	103.75±3.16
SMX/TMP (11.2/2.3 mg/kg)	105.75±0.75	110.50±2.63	150±2.39*	160.50±4.79*
LPV/r (11.4/2.9 mg/kg)	107.00±1.35	111.00±1.78	113.75±1.11	162.75±2.69*
SMX/TMP+LPV/r	118.50±1.04	119.00±1.08	122.50±2.53*	170.75±1.97*

Results are express as mean±SEM, n=5, *Means significant difference with respect to the control at p<0.05 analysis of variance, SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/ritonavir

and 8 was observed in animals co-administered SMX/TMP+LPV/r with respect to the control (Table 2).

Treatment with SMX/TMP produced a time-dependent increase in serum urea, i.e., from the control value 4.15±0.06 to a significant value 6.48±0.09 in week 8 which represents 37% increase when compared with the control (Table 3). Animals treated with LPV/r produced a time-dependent increase in serum urea but significant (p<0.05) increase occurred in week 6 and 8 of treatment with respect to the control (Table 3). Significance (p<0.05) time-dependent elevation in serum urea was observed in animals treated with SMX/TMP+LPV/r in week 6 and 8 with respect to the control. Treatment with SMX/TMP did not produce any significant (p>0.05) change in serum uric level with respect to the control. Similar observation was noted in animals exposed to LPV/r and combine doses of SMX/TMP+LPV/r with respect to the control (Table 3).

Effects on MDA and SOD

Treatment with SMX/TMP produced a time-dependent increase in MDA level, but increase becomes significant only in week 8 with respect to the control (Table 5). Significant (p<0.05) increase in MDA level was noted in animals exposed to LPV/r, i.e., from the control value 44.6±0.16 to 68.5±2.26 in week 8. Co-administration of SMX/TMP+LPV/r produced a time-dependent increase in kidney MDA level with significant (p<0.05) in week 6 and 8, respectively, with respect to control (Table 5). Decrease in kidney SOD was noted in animals exposed to LPV/r which is significant in week 8 with respect to the control (Table 6). Treatments with SMX/TMP and combine doses of SMX/TMP+LPV/r pro duced a time-dependent decrease in tissue SOD levels which become significant in week 8 when compared with the control value (Table 6).

Effect on histopathology of the kidney

The histopathological effects of SMX, LPV/r, and their combination on the kidney structure of animals treated for 8 weeks are shown in Figures 1-4. The kidney of animals in the control group treated with 1% ethanol shows normal histological structure with normal glomeruli, tubules, and interstitium (Figure 1). Kidney of animals treated with LPV/r (11.4/2.9 mg/kg) for 8 weeks shows normal glomeruli, with few others showing hypercellarity of the interstitium of glomeruli (Figure 2). Kidney of animals treated with combine doses of SMX/TMP+LVP/r shows normal glomeruli in number and structure with few others showing hypercellarity of the interstitium of the glomeruli and vascular congestion (Figure 4).

Table 3: Effects of LPV/r, SMX/TMP, and their combination on serum urea (mmol/L) in rats.

Dose	Week 2	Week 4	Week 6	Week 8
Control	4.15±0.06	4.13±1.07	4.14±2.36	4.15±5.16
SMX/TMP (11.2/2.3 mg/kg)	4.20±1.07	4.55±2.36	6.12±2.15*	7.48±0.09*
LPV/r (11.4/2.9 mg/kg)	4.28±0.13	4.65±3.06	7.60±2.11*	8.48±0.09*
SMX/TMP+LPV/r	4.50±1.24	4.72±2.26	8.30±0.11*	10.28±3.16*

Results are express as mean±SEM, n=5, *Means significant difference with respect to the control at p<0.05 analysis of variance. SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/ritonavir

Table 4: Effects of LPV/r, SMX/TMP, and their combination on serum uric acid (mg/L) in rats.

Dose	Week 2	Week 4	Week 6	Week 8
Control	1.35±1.17	1.36±1.36	1.35±3.16	1.37±1.26
SMX/TMP (11.2/2.3 mg/kg)	1.35±0.07	1.34±0.06	1.35±0.05	1.133±0.09
LPV/r (11.4/2.9 mg/kg)	1.36±0.13	1.37±0.06	1.36±0.11	1.35±0.09
SMX/TMP+LPV/r	1.35±0.04	1.33±0.06	1.36±0.11	1.37±0.06

Results are express as mean±SEM, n=5, SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/ritonavir

Table 5: Effects of LPV/r, SMX/TMP, and their combination on kidney MDA (nmole/g tissue) in rats.

| Dose	Week 2	Week 4	Week 6	Week 8
Control	44.5±2.16	45.3±0.17	43.2±2.36	44.6±0.16
SMX/TMP (11.2/2.3 mg/kg)	44.0±2.I5	45.3±2.14	46.0±0.07	60.5±1.26*
LPV/r (1.4/2.9 mg/kg)	45.0±1.08	47.8±2.13	47.0±2.14	68.5±2.26*
SMX/TMP+LPV/r)	45.9±3.14	46.5±3.46	53.8±2.16*	70.5±1.07*

Results are express as mean±SEM, n=5, *Means significant difference with respect to the control at p<0.05 analysis of variance, SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/ritonavir, MDA: Malondialdehyde
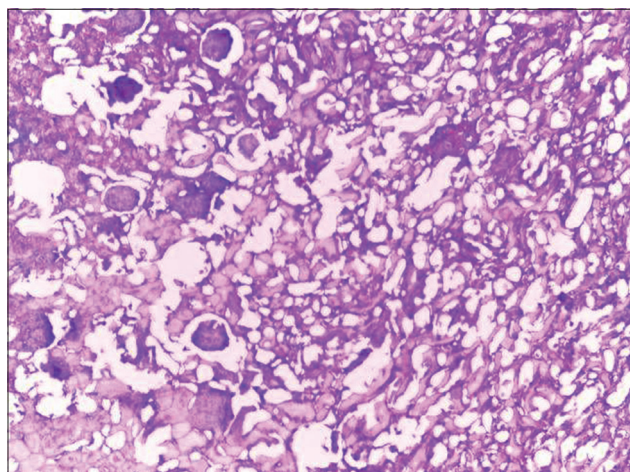
Table 6: Effects of LPV/r, SMX/TMP, and their combination on kidney SOD (units/g protein) in rats.

Dose	Week 2	Week 4	Week 6	Week 8
Control	10.2±2.15	10.2±3.26	10.2±1.25	10.2±2.20
SMX/TMP (11.2/2.3 mg/kg)	10.32±1.15	9.50±2.06	8.60±0.11	5.53±0.08*
LPV/r (1.4/2.9 mg/kg)	9.82±2.07	9.52±1.06	8.43±1.05	5.00±1.17*
SMX/TMP+LPV/r	9.0±2.02	8.53±1.01	7.91±0.86	4.02±1.07*

Results are express as mean±SEM, *Means significant difference with respect to the control at $p<0.05$ analysis of variance. SEM: Standard error of the mean, SMX/TMP: Sulfamethoxazole/trimethoprim, LPV/r: Lopinavir/Ritonavir, SOD: Superoxide dismutase
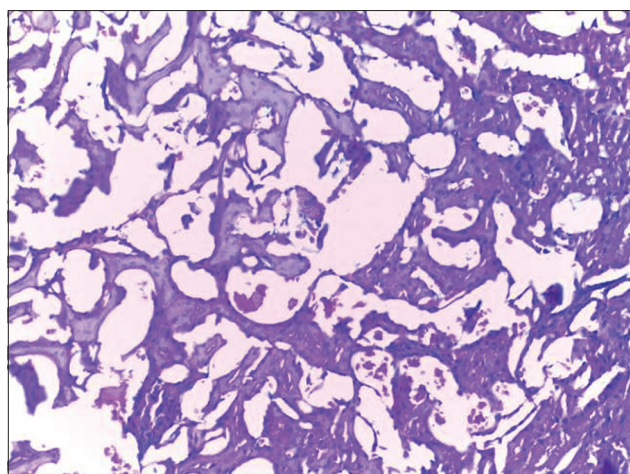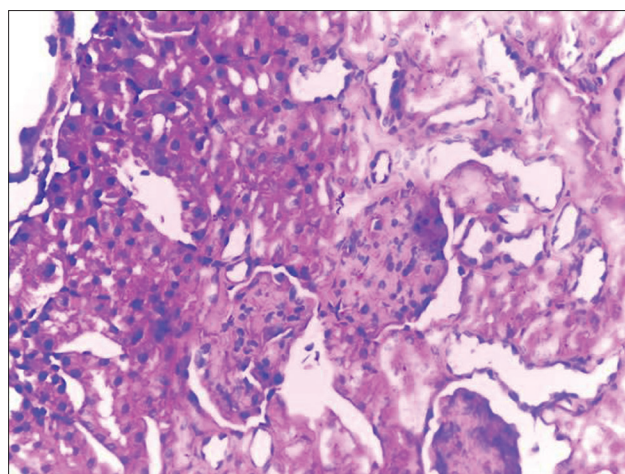

Figure 1: This is the photomicrograph of the hematoxylin and eosin stained section of the kidney of control animal treated with 1% ethanol showing normal glomeruli, tubules, and interstitium (×400).
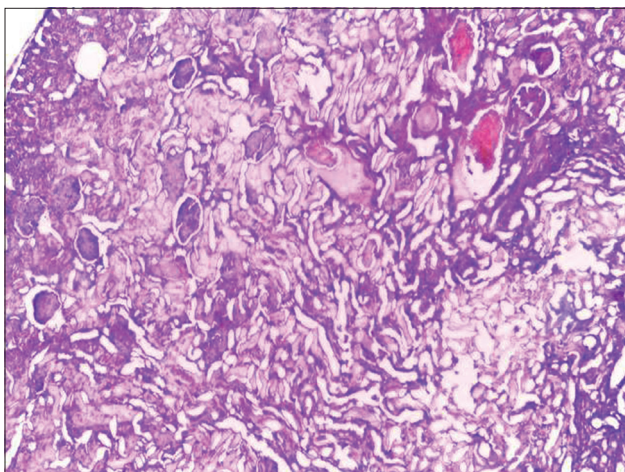

Figure 3: This is the photomicrograph of the hematoxylin and eosin stained section of the kidney of animals treated with sulfamethoxazole/trimethoprim (11.2/2.3 mg/kg) for 8 weeks. It shows normal glomeruli in number and structure with few others showing hypercellarity of the interstitium of the glomeruli (×400).

Figure 2: This is the photomicrograph of the hematoxylin and eosin stained section of the kidney of animals treated with lopinavir/ritonavir (11.4/2.9 mg/kg) for 8 weeks. It shows normal glomeruli in number and structure with few others showing hypercellarity of the interstitium of the glomeruli (×400).

Figure 4: This is the photomicrograph of the hematoxylin and eosin stained section of the kidney of animals treated with sulfamethoxazole/trimethoprim+lopinavir/ritonavir for 8 weeks. It shows normal glomeruli in number and structure with few others showing hypercellarity of the interstitium of the glomeruli and vascular congestion (×400).

DISCUSSION

It is known that 3-4% of drugs and chemicals are eliminated by the kidneys in rats. The kidney has a high blood flow that exposes renal parenchyma to high-peak concentrations of drugs, even if they are only present transiently in the circulation.[20] Due to the high rate of blood flow through kidney, and consequently, the high level of toxins it has to process, the kidney is at risk of developing drug-related

damage. Studies focusing on the correlation between humans and animal have suggested that renal changes in the rat often correctly predict clinical effects on the urinary system in humans, with a modest degree of over prediction.[20] Drug-induced kidney damage could be mediated through quite a number of factors including interactions between co-administered drugs which is common in HIV/AIDS management due to co-morbidity and co-infection.[21] This study evaluates the possible interaction between the co-administration of LPV/r and SMX/TMP on kidney function and structure of albino rats. Effects on the serum creatinine and serum urea were evaluated because they are known biomarkers of kidney function[22] while MDA and SOD are used as markers of chemical agents induced oxidative stress in tissues and organs.[23]

Increase in serum urea and creatinine observed in animals exposed to SMX/TMP is a marker of the renal toxicity due to reports that established creatinine as a fundamental marker of kidney function.[24] The ability of SMX/TMP to increase serum creatinine level agrees with reported observation.[25,26] Increase in serum urea and creatinine observed in animals administered LPV/r can be correlated with similar observations reported by some researchers.[27,28] LPV/r belongs to the protease inhibitors family and reports have linked some members in this family with urologic or renal side effects such as acute renal failure, chronic renal failure, and mild proteinuria.[29] Lack of synergistic effects on serum creatinine, urea, and uric acid observed in animals co-administered with SMX/TMP+LPV/r shows that concurrent use of these drugs may be safe on the renal system.

Malondialdehyde is the major oxidative product of the oxidation of polyunsaturated fatty acids.[30,31] Thus making an increased in kidney MDA level an important indicator of lipid peroxidation and oxidative stress.[32] Increase in MDA observed in this study after treatment with single agents is a marker of lipid peroxidation due to oxidative stress. Kidney tissue contains antioxidant enzymes which include SOD to protect itself from the hazardous effects of oxidative attack; decrease in kidney SOD is a marker of tissue injury.[33,34] Decrease in SOD after treatment with these single agents is a marker of kidney injury. Based on observations in this study, the concurrent use of these agents may not connotes danger to the renal system, because their co-administration produced no synergistic effects on kidney MDA and SOD.

Mild histopathological changes which include hypercellarity of the interstitium of the glomeruli with respect to SMX/TMP treatment for 8 weeks is in agreement with the work of Mozaffari and Rashidi[35] who reported interstitial nephritis in kidney of rats treated with 150 mg/kg of SMX+30 mg/kg TMP. Cunha *et al.*[36] evaluated the toxicological effect of LPV/r on rats, and they reported morphological changes in kidney which is consistent with the observation in this study. Concurrent use of these agents may not portend danger to the renal system to due lack of synergistic histopathological

changes in the kidney of animals treated with combined doses of these agents.

Antiviral and other drugs induce kidney injury can occur through, at least three pathways. One of the pathways is 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 could lead to tubular cell toxicity.[37,38] Another pathway which can result in programmed cell death is the activation of the mitogen-activated protein kinase pathway which can affect barrier function in renal epithelial cell cascade.[39] Other pathways could be associated with antiviral-induced kidney injury is the production of reactive oxygen species which can damage mitochondria, disrupting fatty-acid oxidation, and energy production.[40]

CONCLUSION

The concurrent use of SMX/TMP+LPV/r in the management of HIV and co-infection may have no deleterious effect on the renal system.

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