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Research Article

Renal effect of atazanavir-ritonavir in pregnant albino rats

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Background: Pregnancy could be associated with renal physiologic changes; hence the use of atazanvair/ritonavir (ATV/r) in pregnant women with human immunodeficiency virus (HIV) could be of safety concern due to its nehprotoxic potential.

Objective: The present study was aimed at assessing the renal profile of atazanavir/ritonavir (ATV/r) in pregnant albino rats.

Methods: Thirty six pregnant albino rats were randomized into six groups (A-E) of n=6. Rats in groups A and B were treated with water and normal saline as placebo and solvent control respectively. Rats in groups C-F were orally treated daily with 4.28/1.43, 8.57/2.86, 17.1/5.7 and 34/11.4 mg/kg of ATV/r for 16 days. Rats were weighed, sacrificed and blood was collected and serum extracted. The serum was evaluated for creatinine (Cr), urea (U), uric acid (UA), total protein (TP), albumin (Ab), potassium (K^+), sodium (Na^+), chloride (Cl^-) and bicarbonate (HCO_3^-). Kidneys were harvested, weighed and evaluated for superoxide dismutase (SOD), catalase (CAT) glutathione (GSH), glutathione peroxidase (GPX) and malondialdehyde (MDA) levels. Kidneys were also evaluated for histological damage.

Results: ATV/r-treated groups did not show significant ($p > 0.05$) changes in the body weight, kidney weight and serum electrolytes, however; serum levels of Cr, U, UA were significantly ($p < 0.05$) increased whereas Ab and TP were significantly ($p < 0.05$) decreased in a dose-dependent manner. Kidney SOD, CAT, GSH and GPX levels were significantly ($p < 0.05$) decreased whereas MDA levels were significantly ($p < 0.05$) increased in a dose dependent manner in ATV/r-treated rats. Varying degrees of histological damage were observed in the kidneys of ATV/r treated rats.

Conclusion: This study observed dose-dependent nephrotoxic effects in ATV/r-treated pregnant albino rats.

Keywords: Atazanavir/ritonavir; Kidney, Toxicity, Pregnant; Rats

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1. Introduction

The introduction of highly active antiretroviral therapy (HAART) has led to a dramatic decline in mortality and morbidity associated with HIV infection, however, a variety of adverse renal effects due to antiretroviral drugs have been recognized (Izzedine et al, 2009). Since the kidney plays a major role in the metabolism and excretion of antiretroviral drugs, it is vulnerable to various types of renal injuries associated with antiretroviral drugs (Kalyesubula and Perazella 2011). Antiretroviral associated kidney injuries occur via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals within renal tubular lumens (Perazella, 2010).

The use of antiretroviral drugs in pregnancy, has decreased vertical transmission rate to less than 2%, delayed disease progression and reduced the risk of HIV transmission to HIV-serodiscordant partners. Although these results are notable, maternal toxicities associated with the use of specific antiretroviral drugs during pregnancy remain a serious concern (Burdge et al, 2003; van Schalkwyk et al, 2008). Renal toxicity could be a serious clinical challenge in the provision of antiretroviral therapy during pregnancy due to the fact that in some cases, pregnancy could be associated with acute kidney injury or acute renal failure (Lang and Jones, 1964). Pregnancy associated acute kidney injury is difficult to diagnose, hard to predict and often diagnosed after significant damage to the kidney (Van Hook, 2014). It is often characterized by deterioration in renal function, resulting in the accumulation of nitrogenous waste products, electrolyte homeostatic aberrations and extracellular volume abnormalities (Kellum et al, 2012). Also, evidence showed that pregnancy may affect the incidence of treatment related adverse effects including adverse renal effects (Clark, 2005).

ATV/r combination belongs to the protease inhibitor family and could be used for the management of HIV in pregnancy. The PIs have shown significant decreases in maternal viral load in HIV positive women when compared to other regimens and could decrease the need for cesarean delivery and intrapartum zidovudine and minimize vertical transmission to infants (Duryea et al, 2015), The use of PI-containing HAART especially ATV/r could be associated with renal toxicity (Chan-Tack et al, 2007; Hamada et al, 2012). ATV/r has the potential to yield its crystal in urine and renal interstitial tissues, leading to crystalluria, urolithiasis, acute kidney injury (AKI) or chronic kidney disease (CKD) (Hara et al, 2015).Therefore use in pregnancy may present an important safety concern because pregnancy is characterized by acute kidney injury, renal physiological alterations, including changes in kidney size as well as glomerular and tubular function (Davison and Dunlop 1980; Odutayo et al, 2012). Therefore, this study assessed the renal profile of ATV/r in a pregnant rat model.

2. Methods

2.1 Drugs

The sample of ATV/r used for this study was produced by Myland laboratories limited, India. All other chemicals used for this study are of analytical grade. This study used 4.28/1.43, 8.57/2.86, 17/5.72, and 34/1 1.4 mg/kg/day of ATV/r which represent clinical dose, 2, 4 and 8 times the clinical dose (Guiard-Schmid et al, 2005). ATV/r used for the study was suspended in normal saline (Youssef et al, 2015). Higher doses of ATV/r were used because antiretroviral drugs are used for life and may accumulate. Also, laboratory animals' metabolize and excrete drugs faster than humans (Lüllmann et al, 1975).

2.2 Animals

Pregnant albino rats of average weight 180 ± 5g used for this study were obtained from the animal house of the Department of Pharmacology and Toxicology Madonna University, Elele, Rivers State. The rats were housed in individual cages 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.*

The reproductive status and estrous period of the rats were determined by obtaining their virginal smears. After two complete regular cycles, timed mating of female rats was done on the night of the pro-estrous (N) phase of the cycle. In the morning following mating, vaginal smears were taken again. The presence of spermatozoa and squamous cells in the smear confirmed mating and fertilization of ovules. The sperm – positive morning was thus designated day 0 of pregnancy.

This study used thirty six (36) pregnant albino rats which were divided into six groups A –F of six rats each. Group A (Placebo control) and Group B (Solvent control) were treated orally with water and normal saline respectively. Groups C – F were treated orally with 4.28/1.4, 8.57/2.86, 17/5.72, and 34/1 1.4 mg/kg of ATV/r for 16 days respectively. All rats were treated from the 1st -16th day of gestation.

2.3 Collection of samples

The rats were sacrificed with the aid of inhalational diethyl ether at the end of drug treatment and blood samples were collected via cardiac puncture in sterile non-heparinized containers. Blood samples were centrifuged at 1500rpm for 20 minutes and serum was extracted and analyzed for renal function parameters.

Kidneys were collected via dissection and washed in an ice cold 1.15% KCl solution. Kidneys were homogenized with 0.1M phosphate buffer (pH 7.2) and centrifuged at 1500rpm for 20 minutes. Supernatant was decanted and evaluated for kidney levels of oxidative stress indices.

2.4 Evaluation of renal function parameters

Creatinine was evaluated using Jaffe's deproteinisation method 1886 while urea was evaluated using diacetyl monoxime method (Okonkwo et al, 2013). Uric acid was evaluated as reported by Sanders et al, 1980. Albumin was evaluated using endpoint method as reported by Tietz et al, (1994) while total protein content was evaluated using Biuret method as described by Plummer, (1971).

Serum potassium and sodium were determined using flame photometric methods, while chloride levels were determined using titrimetric method. Bicarbonate was evaluated using standard laboratory test kits.

2.5 Oxidative stress marker assay

Superoxide dismutase activity was evaluated as reported by Sun and Zigman 1978 while catalase was evaluated as reported by Aebi 1984. Reduced glutathione was analyzed according to Sedlak and Lindsay 1968 while glutathione peroxidase was evaluated as described by Rotruck et al, 1973. Malondialdehyde was evaluated as reported by Buege and Aust, 1978.

2.6 Histopathological examination

Collected kidney was fixed in 10% formalin in labeled bottles. Kidney tissues were processed routinely and embedded in paraffin wax. Sections of 5 µ thickness were cut, stained with haematoxylin and eosin and examined under a light microscope.

2.7 Statistical analysis

Results were expressed as mean ± SD. The data were subjected to one way analysis of variance (ANOVA) test and Dunnett's multiple range post-hoc test. Results were considered to be significant at $p<0.05$.

3. Results

Treatment with ATV/r did not produce significant ($p>0.05$) effects on the body, absolute and relative kidney weights when compared to placebo control (**Table 1**).

In **Table 2**, the present study observed significant ($p<0.05$) and dose-dependent increases in serum creatinine (Cr), urea (U), and uric acid (UA). On the other hand, significant ($p<0.05$) and dose-dependent decreases were observed in total protein (TP) and albumin (Ab) levels when compared to the levels of these parameters in the placebo control rat. Cr levels were increased by 41.8%, 87.3%, 154.5%, and 276.4%, whereas UA levels were increased by 52.6%, 97.3%, 170.6%, and 321.0%. The observed increases in U represent 42.1%, 94.7%, 161.4% and 222.6% at 4.28/1.43mg/kg, 8.57/2.86mg/kg, 17/5.72mg/kg, and 34/1 1.4 mg/kg of ATV/r, respectively.

Furthermore, treatment with ATV/r did not produce significant ($p>0.05$) effects on serum K^+, Na^+, Cl^- and HCO_3^- when compared to control (**Table 3**). However, kidney levels of GSH, CAT, SOD and GPX were decreased while MDA levels were increased significantly ($p<0.05$) and in a dose dependent manner when compared to control. GSH levels were decreased by 14.6 %,, 35.0%, 55.7%, and 75.0% while GPX levels were decreased by 13.3 %, 34.0%, 57.3%, and 74.5% at 4.28/1.43mg/kg, 8.57/2.86mg/kg, 17/5.72mg/kg, and 34/1 1.4 mg/kg of ATV/r respectively. Also, SOD levels were decreased by 13.2%, 36.1%, 51.1%, and 70.6%, whereas CAT levels were decreased by 12.7 %, 39.9%, 63.8%, and 99.9%, at 4.28/1.43mg/kg, 8.57/2.86mg/kg, 17/5.72mg/kg, and 34/1 1.4 mg/kg of ATV/r respectively (**Table 4**).

Table1: Effects of atazanavir-ritonavir on body and kidney weights of pregnant albino rats

Groups	Body weight (g)	Absolute kidney weight(g)	Relative kidney weight (%)
A (placebo)	250±12.4	0.97±0.08	0.39 ±0.06
B (solvent)	245±12.9	0.90±0.07	0.37 ±0.09
C	230±10.5	0.93±0.03	0.40 ± 0.01
D	240±11.7	0.99±0.04	0.41 ± 0.08
E	245±10.8	1.03±0.06	0.42 ± 0.03
F	252±13.1	1.00±0.08	0.40 ± 0.08

ATV=Atazanavir-ritonavir. Data are expressed as mean± SD. n=6

Table 2: Effects of atazanavir-ritonavir on serum renal function parameters of pregnant albino rats

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Albumin (mg/dL)	T. Protein (mg/dL)
A(placebo)	$1.10±0.03^a$	$33.3±3.14^a$	$1.14±0.05^a$	$5.98±0.15^a$	$9.79±0.27^a$
B (solvent)	$1.21± 0.01^a$	$31.0±2.00^a$	$1.10±0.03^a$	$5.70±0.41^a$	$9.65±0.51^a$
C	$1.56±0.05^b$	$50.8±3.11^b$	$1.62±0.04^b$	$4.85±0.25^a$	$8.66±0.29^a$
D	$2.06±0.04^c$	$65.7±1.76^c$	$2.22±0.09^c$	$3.53±0.04^b$	$5.31±0.10^b$
E	$2.80±0.06^d$	$90.1±6.85^d$	$2.98±0.08^d$	$2.25±0.08^c$	$3.14±0.03^c$
F	$4.14±0.08^e$	$140.2±10.1^e$	$3.70±0.15^e$	$1.00±0.69^d$	$1.04±0.01^d$

ATV=Atazanavir-ritonavir. n=6. Data are expressed as mean± SD. Values with different superscript on the same column differ significant at ($P<0.05$) ANOVA and Dunnett's multiple range post-hoc test.

Table 3: Effect of atazanavir-ritonavir on serum electrolytes of pregnant albino rats

Groups	K^+ (mEq/L)	Na^+ (mEq/L)	Cl^- (mEq/L)	$HC03^-$ (mEq/L)
A(placebo)	4.33± 0.06	137.2± 6.25	97.3 ± 5.00	29.9± 3.22
B (solvent)	4.45 ± 0.07	140.3 ± 7.11	90.1 ± 7.21	30.6± 4.51
C	4.24 ± 0.01	138.3 ± 9.14	96.6 ± 4.01	32.1± 2.01
D	4.22 ± 0.03	130.0± 5.24	96.1 ± 4.08	30.9± 2.34
E	4.20 ± 0.05	133.2 ± 4.75	94.7 ± 5.11	31.6± 2.72
F	4.15 ± 0.03	128.6± 6.20	89.3± 6.73	33.0± 2.54

ATV=Atazanavir-ritonavir. Data are expressed as mean± SD. n=6

Table 4: Effect of atazanavir-ritonavir on kidney oxidative stress indices of pregnant albino rats

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/mg protein)	GPX (µg/mg protein)
A(placebo)	0.55±0.03^a	21.9±1.06^a	25.3±1.22^a	15.8±0.24^a	18.0±0.97^a
B(solvent)	0.53± 0.06^a	20.1±2.00^a	24.9±2.11^a	15.6±0.17^a	18.5±0.65^a
C	0.62±0.02^a	19.0±1.44^a	22.1±0.77^a	13.5±0.62^a	15.6±0.41^a
D	0.78±0.02^b	14.0±1.51^b	15.2±0.12^b	10.25±0.39^b	11.8±0.54^b
E	1.00±0.02^c	10.7±1.30^c	7 .17±1.41^c	7.00±0.66^c	7.69±0.63^c
F	1.35±0.57^d	6.43±1.67^d	4.01±0.19^d	3.80±0.48^d	4.05±0.47^d

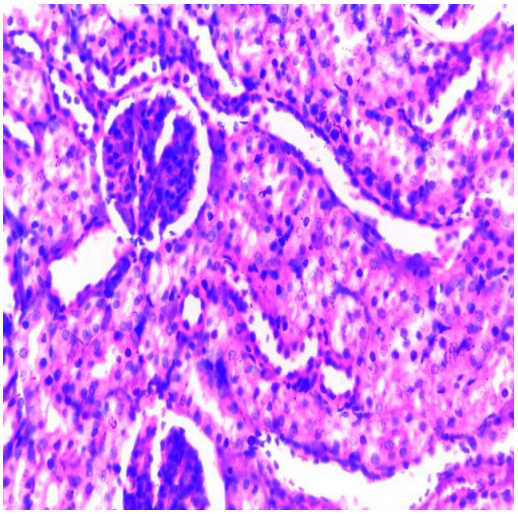
ATV=Atazanavir-ritonavir. n=6. Data are expressed as mean± SD. Values with different superscript on the same column differ significant at ($P<0.05$) ANOVA and Dunnett's multiple range post-hoc test

The kidney of the control rat showed normal histology also, the kidney of pregnant rat treated with4.28/1.43 mg/kg showed normal histology. However, the kidney of rat treated with 8.57/2.86 mg/kg of ATV/r showed tubular necrosis and sloughing of epithelial lining, whereas the kidney of rat treated with 17.1/5.74 mg/kg of ATV/r showed tubular necrosis and glomerular collapse. The kidney of rat that received 32.4/11.4 mg/kg of ATV/r showed tubular necrosis, enlarged tubular epithelial cells and eosinophilic cytoplasm (**Figure 1:A-E**).

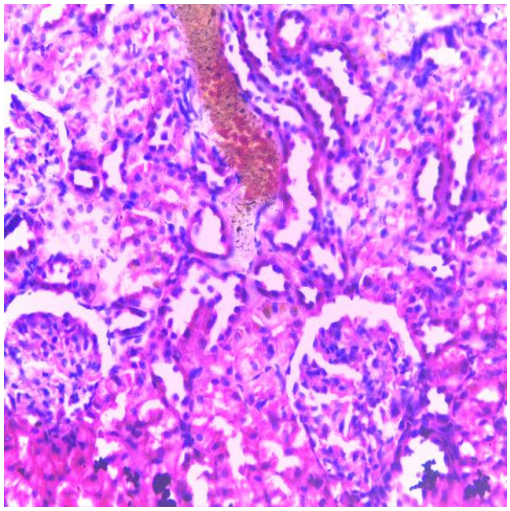
4.0 Discussion

Renal toxicity is an important concern in the provision of antiretroviral therapy in pregnancy. Renal injury associated with highly active antiretroviral therapy (HAART) include electrolyte imbalance to more serious incidents with acute renal failure (Atta et al, 2008).Also, pregnancy could be associated with acute kidney injury (Grünfeld and Pertuiset, 1997; Krane et al, 1998) which might be aggravated with the use of antiretroviral drugs. The present study assessed the renal profile of ATV/r in pregnant albino rats. The organ to body weight ratio gives a proportional size of the organ to body weight. It has been suggested that the use of organ/body weight ratio may be valuable in evaluating the relationship between certain experimental situations and the biological response of a test organism (Wilber et al, 1965). In this study, treatment with ATV/r did not produce significant effects on the body, absolute and relative kidney weights of pregnant rats.

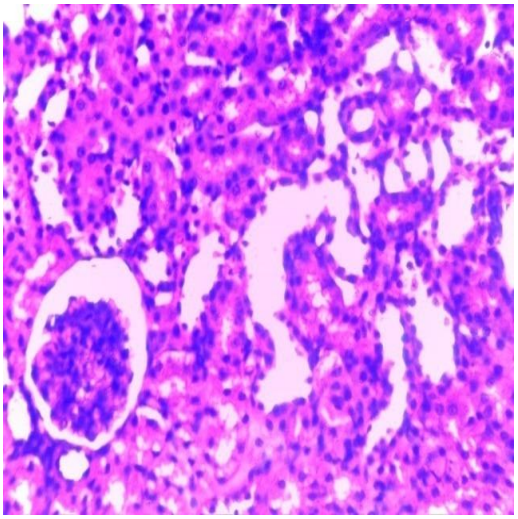
Creatinine is synthesized primarily in the liver from the methylation of glycocyamine by S-adenosyl methionine and is removed from the blood chiefly by the kidneys, via glomerular filtration and proximal tubular secretion (Gross et al, 2005; Allen et al, 2012). Uric acid is the end product of purine metabolism in humans which is filtered out of the blood by the kidney (Young et al, 1987). Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and is filtered out of blood by kidney glomeruli (Corbett, 2008). Serum levels of creatinine, urea and uric acid are used as clinical end points to assess renal function. The current study observed increases in the serum levels of creatinine, urea and uric acid in a dose-dependent manner in ATV/r-treated pregnant rats. The observed low clearance of creatinine, urea and uric acid indicates impaired ability of the kidneys to filter these waste products from the blood and excrete them in the urine. Hence, the elevated blood levels of creatinine, urea and uric acid are diagnostic of impaired renal function (Pagana et al, 1998). Quantification of serum protein is now a central part of screening and monitoring of kidney disease. Measurement of albumin level is one of the most prognostically significant biomarkers of kidney disease outcome and even cardiovascular disease and death (van der Velde et al, 2011).



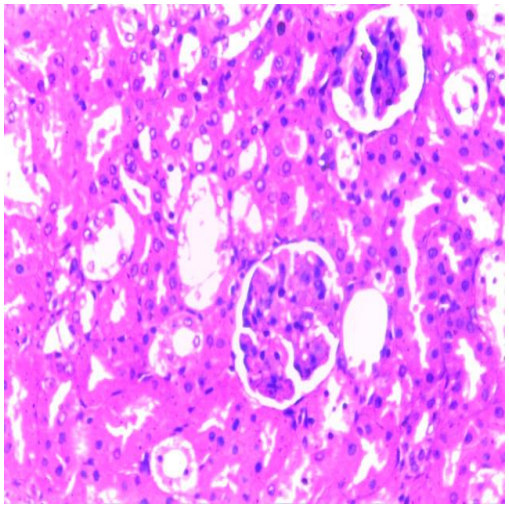
A: Kidney of control pregnant rat showing normal histology (H&E stain, x400)



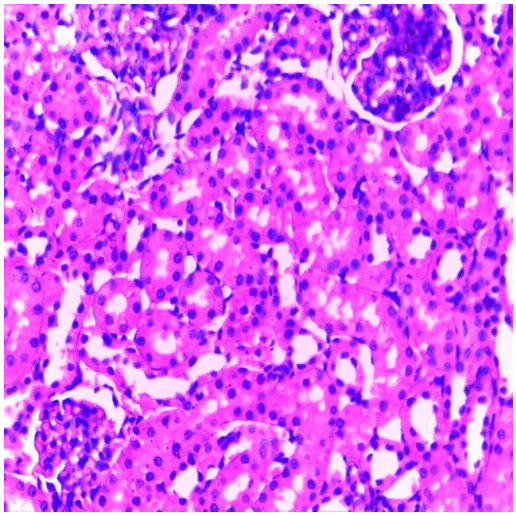
B: Kidney of pregnant rat treated with 4.28/1.43 mg/kg showing normal histology (H&E stain, x400)



C: Kidney of rat treated with 8.57/2.86 mg/kg of ATV/r showing sloughing of tubular epithelial lining and tubular necrosis (H&E stain, x400)



D: Kidney of rat treated with 17.1/5.74 mg/kg of ATV/r showing tubular necrosis and glomerular collapse (H&E stain, x400)



E: Kidney of rat treated with 32.4/11.4 mg/kg of ATV/r showing tubular necrosis, enlarged epithelial cells and eosinophilic cytoplasm (H&E stain, x400)

Figure 1: Kidney photomicrographs showing kidney histology of treated and control rats

In the current study, assessment of serum albumin and total protein levels in ATV/r-treated pregnant rats showed dose-dependent decreases. This observation could be attributed to increase in the renal excretion of TP and Ab because renal pathology characterized by persistent urinary protein and albumin is a burdensome complication associated with long-term ATV/r use (Rockwood et al, 2011).

K^+, Na^+, Cl^- and HCO_3^- which are serum electrolytes found in the body's cells and intracellular fluid perform essential regulatory functions. K^+ along with glycogen plays a key role in transporting glucose into the muscle cells (Armstrong et al, 1985). Na^+ and K^+ are the co-factor for the K^+/Na^+ ATPase activity (Ambwani et al, 1999). Also, K^+ interacts with Na^+ and Cl^- to control fluid and electrolyte balance and assists in the conduction of nerve impulse (Brouns, 1992). Considering the importance of electrolytes in body physiology, evaluation of K^+, Na^+, Cl^- and HCO_3^- is often done for both diagnosis and management of renal, endocrine, acid-base, water balance and many other conditions (Gowda et al, 2010). In the present study, serum levels of K^+, Na^+, Cl^- and HCO_3^- were not significantly altered in ATV/r- treated pregnant rats.

Malondialdehyde (MDA) is a highly reactive dialdehyde formed from the reaction of lipid hydroperoxide (LOOH) with alkoxyl radical (LO•). It is used as an index for lipid peroxidation and is associated with free radical production (Vancini et al, 2005). This study observed dose-dependent increases in the kidney levels of MDA in ATV/r-treated rats which indicate lipid peroxidation. SOD, CAT, GSH and GPX are integral components of antioxidant defence against oxidative radicals (Leeuwenburgh and Heinecke, 2001). SOD catalyzes the dismutation of superoxide to hydrogen peroxide, CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen while reduced glutathione (GSH) chemically detoxifies hydrogen peroxide and forms oxidized glutathione (GSSG) (Khan et al, 2010) . Studies have shown that decreases in the levels of these antioxidants are pointers to free radical production and oxidative stress (Ansari, 2010; Omotayo et al, 2010). In the current study, dose-dependent decreases in the kidney levels of SOD, CAT, GSH and GPX were obtained in ATV/r-treated pregnant rats.

The observed depletion in antioxidant levels could be attributed to ATV/r- induced oxidative stress through the generation of oxidative radicals in the kidneys of pregnant rats. Furthermore; the kidneys of the rats treated with the clinical dose of ATV/r showed normal kidney histology, however, histopathological alterations characterized by tubular necrosis were observed in the kidneys of rats treated with higher doses of ATV/r. In the present study, observed histopathological changes correlate with alterations in serum biochemical parameters and kidney oxidative stress indices. The mechanism of ATV/r-induced nephrotoxicity is yet unknown, however, a number of mechanisms involved in antiviral-induced kidney injuries have been proposed. These include the overexpression or competitive inhibition of transport pumps which could lead to tubular cell toxicity (Cihlar et al, 1999; Ho et al, 2000), activation of the mitogen-activated protein kinase pathway which can stimulate programmed cell death (Cihlar et al, 2002) and oxidative stress via damage mitochondria stimulating reactive oxygen species production which can disrupt fatty-acid oxidation and energy production (Cihlar et al, 2002; Saumoy et al, 2004).

5.0 Conclusion

This study was able to show that treatment with ATV/r produced dose-dependent nephrotoxic effects in pregnant albino rats.

Conflict of Interest declaration

The authors declare no conflict of interest.

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