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Effect of rifampicin on the kidney of albino rats

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ABSTRACT: The study was designed to investigate the effect of rifampicin on the kidney biomarkers and histopathology of kidney of albino rats. 42 albino rats were used and rifampicin was administered at 1.10mg/120gBW and 0.55mg/120gBW for intervals of 20, 40 and 60days.Animals were sacrificed 24 hours after the last day of administration and blood samples were collected for the estimation of urea and creatinine levels sodium , potassium, chloride and bicarbonate ion concentrations. Result from this study indicates that urea and creatinine levels in a dose and time dependent manner, significantly increased ($p<0.05$) all through the duration of the study when compared with the control value. Significant decrease ($p<0.05$) was observed for sodium ion concentration in a dose and time dependent manner also while potassium, chloride and bicarbonate ions were non significantly changed ($p>0.05$). Histological examination of the kidney revealed an inflammation of the glomeruli for 1.10mg/120gBW treated group in 60days. In conclusion, prolonged treatment of tuberculosis patients with rifampicin could result to nephrotoxicity.© JASEM

KEY WORDS: Rifampicin Tuberculosis, Urea, creatinine, Sodium, Potassium, Chloride, Bicarbonate

Tuberculosis (TB) is an infectious disease caused by bacteria whose scientific name is *Mycobacterium tuberculosis.* It was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery. TB most commonly affects the lungs but also can involve almost any organ of the body. The main cause of Tuberculosis, *Mycobacterium tuberculosis* (MTB), is a small aerobic non-motile bacillus. High lipid content of this pathogen accounts for many of its unique clinical characteristics (Southwick, 2007). The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence. The *M. tuberculosis* complex includes four other TB-causing mycobacteria: *M. bovis*, *M. africanum, M. canetti* and *M. microti*. (Van *et al*, 1997) *M. africanum* is not widespread, but in parts of Africa it is a significant cause of tuberculosis.(Niemann *et al*, 2002*) M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has largely eliminated this as a public health problem in developed countries.(Kumar *et al*, 2007) *M. canetti* is rare and seems to be limited to Africa, although a few cases have been seen in African emigrants (Pfyffer *et al.*,1998). *M. microti* is mostly seen in immunodeficient people, although it is possible that the prevalence of this pathogen has been underestimated (Niemann, 2000).

Tuberculosis has been present in humans since antiquity. The earliest unambiguous detection of *Mycobacterium tuberculosis* is in the remains of bison dated 18,000 years before the present. Whether tuberculosis originated in cattle and then transferred to humans, or diverged from a common ancestor infecting a different species, is currently unclear. However, it is clear that *M. tuberculosis* is not directly descended from *M. bovis*, which seems to have evolved relatively recently.

Skeletal remains from a Neolithic Settlement in the Eastern Mediterranean show prehistoric humans (7000 BC) had TB, and tubercular decay has been found in the spines of mummies from 3000–2400 BC.(Zink *et al.*, 2003) Phthisis is a Greek term for tuberculosis; around 460 BC, Hippocrates identified phthisis as the most widespread disease of the times involving coughing up blood and fever, which was almost always fatal(Hippocrates, 2006). In South America, the earliest evidence of tuberculosis is associated with the Paracas-Caverna culture (circa 750 BC to circa 100 AD) (Konomi *et al.*, 2002). Suzanne Austin Alchon wrote that, "Skeletal remains from prehistoric North America indicate that the disease was so common that "virtually every member of these late prehistoric communities had primary exposure to tuberculosis (Austin, 2003). Tuberculosis symptoms include: cough that is worse in the morning (sometimes with hemoptysis, blood in

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the sputum), chest pain, breathlessness, night sweats, and signs of pneumonia. In advanced disease, there may be extreme weight loss. Examination with a stethoscope may reveal diminished breath sounds, bronchial breathing, tracheal deviation, and coarse crackles. Tuberculosis (TB) is divided into two categories: pulmonary and extrapulmonary. Types of pulmonary TB are primary tuberculosis pneumonia, tuberculosis pleurisy, cavitary tuberculosis, miliary TB, and laryngeal tuberculosis. Extrapulmonary tuberculosis occurs primarily in immune compromised patients, examples are lymph node disease, tuberculosis peritonitis, tuberculosis pericarditis, osteal tuberculosis, renal tuberculosis, adrenal tuberculosis and tuberculosis meningitis.
Globally, the World Health Organisation estimates that the largest number of new TB cases in 2008 occurred in the South-East Asia Region, which accounted for 35% of incident cases globally. However, the estimated incidence rate in sub-Saharan Africa is nearly twice that of the South-East Asia Region with over 350 cases per 100 000 population. An estimated 1.7 million people died from TB in 2009. The highest number of deaths was in the Africa Region. In 2008, the estimated per capita TB incidence was stable or falling in all six WHO regions. However, the slow decline in incidence rates per capita is offset by population growth. Consequently, the number of new cases arising each year is still increasing globally in the WHO regions of Africa, the Eastern Mediterranean and South-East Asia. Nigeria has the world's fourth largest tuberculosis (TB) burden, with more than 460,000 estimated new cases in 2007. According to the World Health Organization's (WHO's) 2009 report on global TB control, 42 percent of the new TB cases in 2007 were sputum smear-positive (SS+). Since 2002, DOTS (the internationally recommended strategy for TB control) coverage has increased rapidly from 55 percent in 2002 to 91 percent 2007, and subsequently, case detection and notification of all forms of TB more than doubled from 38,628 in 2002 to 86,241 in 2006. Although still far short of WHO's target of 70 percent, the TB case detection rate increased from 11 percent in 2002 to 23 percent in 2007. After declining for several years, the treatment success rate has stabilized at 76 percent. Both the case detection and treatment success rates were among the lowest of high-burden TB countries. The public health burden posed by TB is becoming increasingly important as the country's HIV/AIDS epidemic unfolds. WHO estimates that more than a quarter of new TB patients are HIV positive. Collaborative TB-HIV/AIDS services are being scaled up and the number of TB patients tested for HIV increased from about 7,500 in 2006 to 27,850 in 2007, or about one-third of all notified cases.

The National TB and Leprosy Control Program (NTBLCP) coordinates and provides strategic direction for TB control activities in Nigeria. The Federal Ministry of Health declared TB a national emergency in April 2006 and inaugurated the National TBHIV/ AIDS Working Group in June 2006 (USAID, 2009).

With the advent of effective antibiotics for TB, drug therapy has become the cornerstone of treatment. Single-drug treatment often causes bacterial resistance to drugs. Therefore, all recommended therapies include multiple drugs given for at least 6 months, often for as long as 9 to 12 months. Adjustments to the treatments are made based on susceptibility of the bacterial strain. A combination of antibiotics is usually prescribed. In 1998, scientists successfully decoded the entire gene sequence, or genome, of the tuberculosis bacteria. This advance is likely to lead to the development of new methods for treatment and prevention of TB.

The World Health Organization (WHO) declared TB a global health emergency in 1993, and the Stop TB Partnership developed a Global Plan to Stop Tuberculosis that aims to save 14 million lives between 2006 and 2015 (WHO, 2011). Since humans are the only host of Mycobacterium tuberculosis, eradication would be possible. This goal would be helped greatly by an effective vaccine (Martin, 2006).

The primary treatment for mycobacteria is specific chemotherapy. For centuries, tuberculosis was a major killer disease but the introduction in the 1960s of rifampicin revolutionized therapy and tuberculosis came to be seen as an easily treated disease. Unfortunately, Mycobacterium tuberculosis has undergone rapid mutations and strains with increased virulence or multidrugs resistance are now common. This has restored tuberculosis to the status of a major health threat (Rang *et at.,* 2007).The first line agents for treatment of tuberculosis are isoniazid (INH), rifampicin, pyrazinamide and ethambutol.

Rifampicin with molecular formula and molecular weight of $C_{43}H_{58}N_4O_{12}$ and 822.96 respectively is ansemisynthetic derivative of rifamycin, an antibiotic produced by *Streptomyces mediterrenei*.It acts by binding to and inhibiting DNA-dependent RNA polymerase in prokaryotic but not in eukaryotic cells.

It enters phagocytics cells hence it can kill tubercle bacilli. (Geo *et al.,*2010).

Rifampicin causes cholestasis at both the sinusoids and canaliculi of the liver because of defect in uptake by hepatocytes and defect in excretion respectively (Haddad, 1983). Rifampicin may produce liver dysfunction. Hepatitis occurs in 1% or less of patients, and usually in the patient with pre-existing liver disease. Hypersensitivity reactions may occur, usually characterized by a "flu" type syndrome. Nephrotoxicity appears to be related to a hypersensitivity reaction and usually occurs after intermittent or interrupted therapy. It has been suggested that some of the adverse effects associated with rifampicin may be attributed to its metabolite diacetyl rifampicin. It is lipid soluble, and thus can reach and kill intracellular, as well as extracellular, Mycobacteria. Rifampicin does not bind to mammalian nuclear RNA polymerase and therefore does not affect the RNA synthesis in human beings. Rifampicin, however, may affect mammalian mitochondrial RNA synthesis at a concentration that is 100 times higher than that which affects bacterial RNA synthesis (Molavi, 1990).

MATERIALS AND METHODS

*Source of experimental animals***:** Forty two (42) albino rats were purchased from the Department of Human Physiology, University of Nigeria Enugu Campus (UNEC) and acclimatized for one week at the Animal House of Biochemistry Department, University of Port Harcourt located at the botanical garden Choba Park. During acclimatization, the animals were fed with rat pellets and water *ad libitum*.

Source of drug: Rifampicin of 600mg used in this study was purchased from NAFDAC approved pharmaceutical store located opposite the gate of University of Port Harcourt Teaching Hospital, Alakahia, Rivers State of Nigeria. The drug details are stated below;
Manufactured by Mekophar chemist pharmaceutical joint stock Co., 29715 Lythiong Kiet street, district 11-Hconc-vietnam,

Marketed by Neros pharmaceutical ltd Batch No: 11007AX , MFD: 29/03/2011 , EXP: 29/03/2014 , REG NO/VISA: VD-1043-06 , NAFDAC REG NO: 04-8420

Chemicals And Reagents: All chemicals and reagents used in this study were of analytical grade.

Equipment.: Centrifuge (Universal 320, Hettich Zentrifugen Germany), refrigerator(Frestech), colorimeter (Jenway 6051 colorimeter; UK), weighing balance (Mettle Toledo. AB 204, Switzerland), Spectrophotometer (Beckman Coulter, DU 520 General Purpose UV / Visual), water bath (UNISCOPE-Sn801A surgifriend medicals, England) and Ion Selective Electrode (ISE) method using Humalyte machine (Human, Germany).

*Preparation of drug solution***.:** 600mg of rifampicin capsule was carefully opened and the powder content emptied into a 500ml beaker. 270ml of distilled water was added and mixed to form a clear solution of concentration of 1.10mg/120gBW . This served as the stock therapeutic solution. From the stock, 20ml was measured into 100ml beaker and made up to 40ml with distilled water to make a concentration of 0.55.mg/120gBW.

Experimental Design: 42 rats were divided into 7 cages (n=6rats). 0.50ml of distilled water was administered to rats in one of the cages which served as control.0.5ml of stock drug solution (1.10mg/120gBW) was administered to rats in three other cages for 20,40 and 60 days, similarly 0.50ml of drug solution (0.55mg/120gBW) to the rats in the three remaining cages for 20, 40 and 60days.

*Collection of blood and preparation of serum***.:** Blood samples were collected 24 hours after 20, 40 and 60days of rifampicin administration.The rat were withdrawn from the cages one after the other anaesthetized and the jugular vein at the neck cut. The blood was put in lithium heparin bottles and spun at 5000rpm using MSE centrifuge to obtain serum for biochemical investigations.

*Biochemical Investigation For Kidney Function***.:** Serum urea was determined by urease enzymatic method, creatinine by Jaffe's reaction and. Serum sodium, potassium, chloride and bicarbonate levels were determined by ion selective electrode(ISE) method using Humalyte machine (Human, Germany) (Tietz 1987).

Histopathogical studies: 10% formalin was freshly prepared and the kidney of treated and control were fixed in 10% formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin (Lillie, 1965).

Statistical Analysis: Results were analyzed using (SPSS) version 15. The data were expressed using descriptive statistics and Analysis Of Variance (ANOVA). Multiple comparisons for the groups were done using Post Hoc Turkey (HSD) to test for the level of significance between means. A $p<0.05$ was considered to be statistically significant. Values are expressed in Means±Standard Deviation (M±SD). Superscript a: Indicates significant difference ($p<0.05$)

RESULT AND DISCUSSION

Result from Tables 1 and 2 showed that urea and creatinine levels in a dose and time dependent manner, significantly increased all through the duration of the study when compared with the control value. Significant decrease was observed for sodium ion concentration in a dose and time dependent manner also from Table 3 while potassium, chloride and bicarbonate ions were non significantly changed as was observed in Tables4,5 and 6. Histological examination of the kidney revealed an inflammation of the glomerulli for 1.10mg/120gBW treated group in 60days as was revealed in Figure 1.

Table 1. Result of the effect of rifampicin on creatinine levels (mmol/L) treated for 20,40 and 60 days**.**

Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	65.65±0.88		
0.55mg/120Gbw	75.17 [a] ±1.14	77.33 [a] ±0.88	81.17 [a] ±0.70
1.10mg/120gBW	80.67 [a] ±1.61	82.67 [a] ±1.12	89.17 [a] ±0.95

Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

Table 2. Result of the effect of rifampicin on urea levels (mmol/L) treated for 20,40 and 60 days.

Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	3.60±0.20		
0.55mg/120gBW	4.40 [a] ±0.15	4.90 [a].±0.07	5.11 [a] ±0.08
1.10mg/120gBW	4.60 [a] ±0.15	5.08 [a] ±0.12	5.22 [a] ±0.12

Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

Table 3. Result of the effect of rifampicin on sodium ion levels (mmol/L) treated for 20,40 and 60 days**.**

Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	141.67±1.03		
0.55mg/120gBW	135.00 [a] ±1.79	137.00 [a] ±0.87	136.83 [a] ±0.98
1.10mg/120gBW	132.33 [a] ±2.16	135.33 [a] ±1.21	136.83 [a] ±0.98

Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

Table 4. Result of the effect of rifampicin on potassium ion levels (mmol/L) treated for 20,40 and 60 days**.**

Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	4.88±0.21		
0.55mg/120gBW	4.83±0.37	4.88±0.17	5.17±0.19
1.10mg/120gBW	4.88±0.37	4.87±0.16	5.00±0.11

Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

Table 5. Result of the effect of rifampicin on chloride ion levels (mmol/L) treated for 20,40 and 60 days.

Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	105.33±1.21		
0.55mg/120gBW	104.33±1.75	105.83±1.83	108.67±1.37
1.10mg/120gBW	105.67±2.16	108.67±3.14	110.67 [a] ±3.20

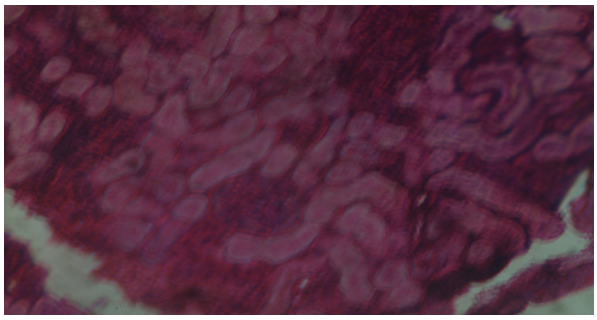
Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

Table 6. Result of the effect of rifampicin on bicarbonate ion levels (mmol/L) treated for 20,40 and 60 days.

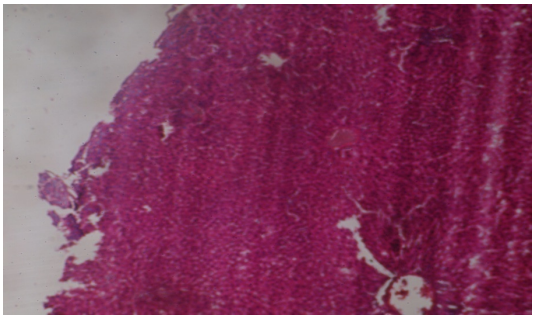
Drug dose / Control.	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
	M±SD		
Control (Distilled water)	20.67±1.03		
0.55mg/120gBW	21.00±1.41	20.17±1.17	19.17±0.75
1.10mg/120gBW	21.33±1.41	20.83±1.17	19.83±1.33

Values are expressed in Means±SD
Superscript a indicates significant difference ($p<0.05$) when compared to control value

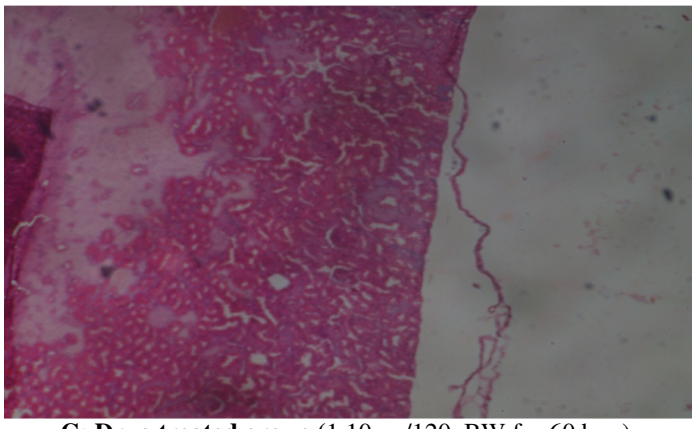
Fig:1:Result of histological changes in the kidney tissues treated with distilled water (control) and1.10mg/120gBW of rifampicin for 20 and 60days. (H & E STAINING).



A: control group treated with distilled water.



B :Drug treated group(1.10mg/120gBW for 20days)



C: Drug treated group (1.10mg/120gBW for 60days)

Figure 1:Result of histological changes in the kidney tissues treated with distilled water (control) and1.10mg/120gBW of rifampicin for 20 and 60days. (H & E STAINING, 400x**).**

Representative photomicrograph of kidney tissues (H&E staining, 400x) showing (A) control-normal, well defined histological structures without any signs of vascular or inflammatory changes, (B)kidney cells after 20 days of treatment-cells normal, well defined histological structures without any signs of vascular or inflammatory changes and (C)kidney cells after 60 days of treatment- inflammation of the glomeruli is evident indicating tissue damage.

Conclusion: The result from this experiment showed that the blood urea concentration (mmol/L) was significantly increased relative to the control value. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract.

The plasma level of creatinine (µmol/L) was also significantly increased relative to the control level. Creatinine is a good biomarker for measuring kidney function. Renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval. Results obtained from this study agree with previous investigations (Salih *et al*, 2008; Keisuke *et al*, 2000).

The histopathology tests indicated glomerulonephritis(Inflammation of the glomeruli) after the sixtieth day of treatment. This observation is in accordance with the investigation of Keisuke *et al.,*(2000) who studied a case of a woman who suffered nephrotic syndrome after four weeks of rifampicin administration. Nader *et al.,* (2001) also presented the case of a 61 year-old alcoholic male who showed features of acute interstitial nephritis after a long-term treatment with rifampicin. Tada *et al.,* (1994). also reported rifampicin-induced minimal change nephrotic syndrome, showing minor glomerular abnormalities and slight interstitial changes. In their patient, electron microscopy showed glomerular changes such as local widening of the subendothelial space which was filled with fine granular or fibrillar materials, irregularity of the endothelial investment, and swelling or shrinkage of the endothelial cells. They speculated that endothelial injury due to rifampicin seemed to play a role in the development of nephrotic syndrome. Rapidly progressive glomerulonephritis associated with nephrotic syndrome was reported in patients treated with rifampicin .Sodium ion concentration significantly decreased indicating inappropriate arginine vasopression secretion or volume depletion secondary to gastrointestinal fluid loss. (upadhyay et al., 2006). However potassium ion non significantly varied.

This study also showed non significant alterations in serum bicarbonate and chloride concentrations. Hypochloremia is frequently observed in cases of metabolic acidosis. Chloride ion concentration is supposed to follow those of Na+ in the absence of acid-base disturbances, but our study observed an increase in Cl- concentration in treated groups. This is a pointer to a possible acid-base disturbance in these patients. Although fluctuations in serum chloride have little clinical consequences, they are signs of an underlying disturbance in fluid and acid-base homeostasis.

Fluid-electrolyte and acid-base derangements are factors for the development of acute renal failure. The changes in Cl- and HCO3 - concentrations albeit small are suggestive of a possible existence of an alteration in the equilibrium of body anions in these patients.

I therefore conclude that renal impairment manifested in form of electrolyte abnormalities and increased urea and creatinine concentrations could result from tuberculosis patients placed on rifampicin drug.

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