



## Effect of rifampicin on the lipid profile of albino rats

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**Key words:** Rifampicin, Tuberculosis, Total cholesterol, High density lipoprotein-cholesterol, triglyceride.

**ABSTRACT:** The study was designed to investigate the effect of rifampicin on the lipid profile and histopathology of the heart of albino rats. Albino rats (42) were used and rifampicin was administered at 1.10mg/120g body weight (BW) and 0.55mg/120g BW for intervals of 20, 40 and 60days. Result from the study revealed that there was a significant increase ( $p<0.05$ ) in triglyceride level for animals dosed with 1.10mg/120g BW -  $1.08\pm 0.07$ ,  $1.37\pm 0.12$  and  $1.52\pm 0.12$  mmol/L for 20, 40 and 60days of drug administration respectively when compared to the control ( $0.78\pm 0.09$  mmol/L). Also there was a significant increase ( $p<0.05$ ) in triglyceride level for those dosed with 0.55mg/120g BW -  $1.20\pm 0.09$ ,  $1.46\pm 0.09$  and  $1.47\pm 0.01$  mmol/L respectively for intervals of 20, 40 and 60days. There was also a significant increase ( $P<0.05$ ) in total cholesterol level for animals dosed with 1.10mg/120g BW ie  $1.85\pm 0.10$ ,  $2.08\pm 0.15$ , and  $2.10\pm 0.18$  mmol/L for 20, 40 and 60 days respectively and animals dosed with 0.55mg/120g BW ie  $1.83\pm 0.15$ ,  $2.03\pm 0.12$  and  $2.05\pm 0.10$  mmol/L for 20, 40 and 60 days respectively when compared to the control ( $1.40\pm 0.06$  mmol/L). There was a significant decrease in the high density lipoprotein-cholesterol level of animals dosed with 1.10mg/120g BW for 20, 40 and 60 days respectively ( $0.37\pm 0.08$ ,  $0.45\pm 0.08$  and  $0.45\pm 0.10$  mmol/L) and also in animals dosed with 0.55mg/120g BW ( $0.40\pm 0.09$ ,  $0.42\pm 0.07$  and  $0.40\pm 0.06$  mmol/L) for 20, 40 and 60 days respectively, when compared to the control value ( $0.68\pm 0.07$  mmol/L) at ( $p<0.05$ ). Histological examination of the heart revealed normal architectural structure of the heart after rifampicin administration for 20 and 60days, though pattern of plasma lipid alteration suggests dyslipidemia; therefore plasma lipid profile should be monitored routinely because of the positive relationship between increased dyslipidemia with cardiovascular diseases. © JASEM

Tuberculosis or TB (short for tubercle *bacillus*) is a common and potentially lethal infectious disease caused by various strains of mycobacteria, usually in humans (kumar *et al.*, 2007). It is caused by a rod shaped bacterium named *Mycobacterium tuberculosis* (MTB). It was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery. Tuberculosis usually attacks the lungs but can also affect other parts of the body (Kumar *et al.*, 2007). It is spread through the air when people who have an active MTB infection cough, sneeze, or otherwise transmit their saliva through the air (Konstantinos, 2010). Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progresses to active disease, which, if left untreated, kills more than 50% of its victims (Konstantinos, 2010). An estimated 1.7 million people died from TB in 2009. The highest number of deaths was in the Africa Region.

Nigeria has the world's fourth largest tuberculosis (TB) burden, with more than 460,000 estimated new

cases in 2007. 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.

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

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National TB -HIV/AIDS Working Group in June 2006. Tuberculosis is the leading cause of death among HIV infected people in Africa. It is estimated that one third of the 40 million people living with HIV/AIDS worldwide are co-infected with TB.

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 multidrug resistance are now common. This has restored tuberculosis to the status of a major health threat (Rang et al., 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 a semisynthetic derivative of rifamycin, an antibiotic produced by *Streptomyces mediterranei*. It acts by binding to and inhibiting DNA-dependent RNA polymerase in prokaryotic but not in eukaryotic cells. It enters phagocytic 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). 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. Rifampicin may affect mammalian mitochondrial RNA synthesis at a concentration that is 100 times higher than that which affects bacterial RNA synthesis (Molavi, 1990).

It has been suggested that some of these adverse effects associated with rifampicin may be attributed to its metabolite diacetyl rifampicin. This is lipid soluble, and thus can reach and kill intracellular, as well as extracellular, Mycobacteria. The effects of rifampicin on lipid profile in albino rats is the aim of this work.

## 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 was Manufactured by Mekophar chemist pharmaceutical joint stock Co., 29715 Lythiong Kiet street, district 11-Hconc-vietnam, and 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 (Mettler Toledo. AB 204, Switzerland), Spectrophotometer (Beckman Coulter, DU 520 General Purpose UV / Visual), water bath (UNISCOPE-Sn801A Surgifriend medicals, England).

*Preparation of drug solution:* Rifampicin (600mg) 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:* The forty-two (42) rats used in this experiment were divided into 7 cages (n=6rats). The control rats were given 0.50ml of distilled water while 0.5ml of stock drug solution (1.10mg/120gBW) was administered to rats in groups 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.

*Method of lipid profile investigation :* The serum total cholesterol, triglycerides (TG) and HDL-cholesterol were determined in sera using commercial kits supplied by Randox (UK). These analyses were carried out using standard techniques.

*Histopathological studies:* 10% formalin was freshly prepared and the heart of treated and control were fixed in 10% formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylene 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 were expressed in Mean ± Standard Deviation (M±SD).

**RESULT AND DISCUSSION**

Results from Table 1, revealed a significant increase in mean values of triglycerides for animals treated with 1.10mg/120g BW of rifampicin after 20, 40 and 60days, likewise those treated with 0.55mg/120g BW when compared to the control value. From Table 2, mean total cholesterol levels of animals dosed with 1.10mg/120g BW and 0.55mg/120g BW for 20, 40, 60days significantly increased when compared to mean control value. There was a significant decrease in the high density lipoprotein-cholesterol level of animals treated with 1.10mg/120g BW and 0.55mg/120g BW of the drug for 20, 40 and 60 days when compared to the control as was observed in Table 3. Histology of the heart tissues showed no morphological changes for 20 and 60days of rifampicin administration from Figure 1.

**Table 1.**Result of the Effect of Rifampicin on Triglyceride levels (mmol/L) treated for 20, 40 and 60 days.

	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
Drug dose / Control.	M±SD		
Distilled water	0.78±0.09		
0.55mg/120gBW	1.20±0.09 <sup>a</sup>	1.46±0.09 <sup>a</sup>	1.47±0.10 <sup>a</sup>
1.10mg/120gBW	1.08±0.07 <sup>a</sup>	1.37±0.12 <sup>a</sup>	1.52±0.12 <sup>a</sup>

Values are expressed in Mean ±SD

Superscript a indicates significant difference (p<0.05) when compared to control value

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

	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
Drug dose / Control.	M±SD		
Distilled water	1.40±0.06		
0.55mg/120gBW	1.83±0.15 <sup>a</sup>	2.03±0.15 <sup>a</sup>	2.05±0.10 <sup>a</sup>
1.10mg/120gBW	1.85±0.10 <sup>a</sup>	2.08±0.15 <sup>a</sup>	2.10±0.18 <sup>a</sup>

Values are expressed in Mean ±SD

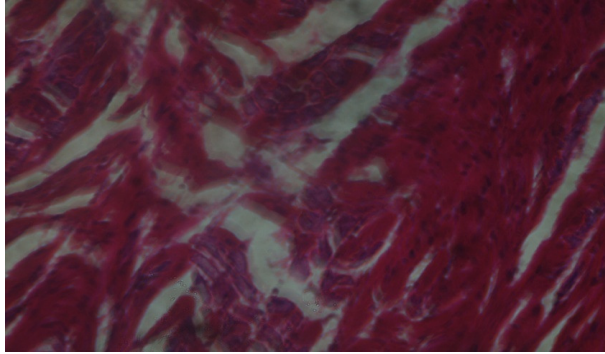
Superscript a indicates significant difference (p<0.05) when compared to control value

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

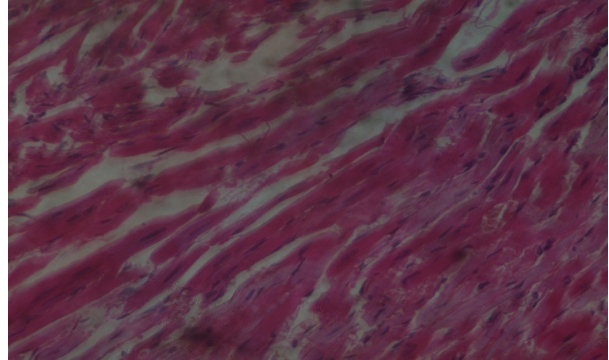
	DAYS OF DRUG TREATMENT		
	20Days	40Days	60Days
Drug dose / Control.	M±SD		
Distilled water	0.68±0.07		
0.55mg/120gBW	0.40±0.09 <sup>a</sup>	0.42±0.07 <sup>a</sup>	0.40±0.06 <sup>a</sup>
1.10mg/120gBW	0.37±0.08 <sup>a</sup>	0.45±0.08 <sup>a</sup>	0.45±0.10 <sup>a</sup>

Values are expressed in Mean ±SD

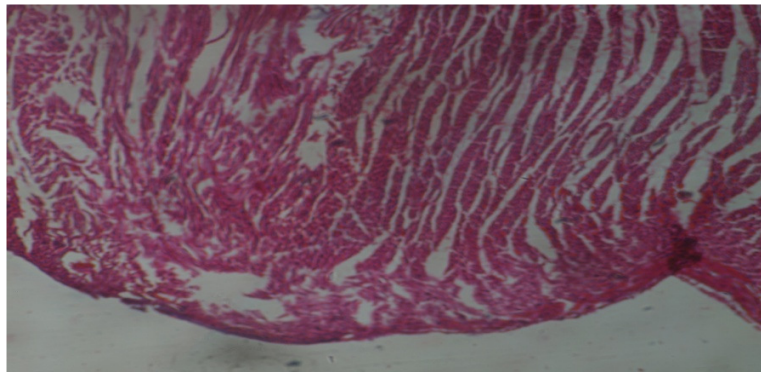
Superscript a indicates significant difference (p<0.05) when compared to control value



**Fig:1:** Result of histological changes of the heart tissue from control group, showing normal architectural features ((400x) H & E staining).



**Fig: 2:** Histology of heart tissue showing normal architectural features of the heart after treatment with 1.10mg/120gBW of rifampicin for 20days ((400x) H & E staining).



**Fig: 3:** Histology of heart tissue showing normal architectural features of the heart after treatment with 1.10mg/120gBW of rifampicin for 60days ((400x) H & E staining).

The result from the study showed that the level of triglyceride increased as the duration of drug administration increased. The same trend was found for total cholesterol though the reverse was the trend for HDL. This trend is supported by report of Chen and Raymond, (2005) that rifampicin inhibits CYPA71, a rate limiting enzyme in the conversion of cholesterol to bile acids'. This may account for the increase in cholesterol levels for when they are not converted to bile acids they remain in the blood stream. Piriou *et al.*, (1979) also reported that fatty liver can be induced by very high doses of rifampicin in rat. The determination of total lipids, total cholesterol and phospholipids showed a significant increase in the total lipids, triglycerides and total cholesterol in the liver at a dose of 400mg. The

increase in plasma triglyceride level may possibly be due to the induction of the activity of regulatory enzymes in the biosynthesis of triglycerides (Faromi *et al.*, 1999). Since lipid metabolism occur in the liver, the alteration of the plasma lipid levels may be related to the fact that hepatotoxicity is a major side effect of rifampicin administration and that rifampicin is an effective liver enzyme inducer. This proposal is in agreement with the suggestion of Provost *et al.*, (2003) that the concentration of circulating triglycerides may be an early and reliable indicator of hepatotoxicity in the rat and its assessment as part of the core list of liver parameters in preclinical studies is recommended

The plasma lipid profile of patients on rifampicin should be monitored routinely because of the positive relationship between increased levels of triglycerides and cholesterol and the reduced level of HDL with cardiovascular disease.

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