Hepatoprotective Role of Neutrosec[R] on Hepatic Damage Induced by Combination of Zidovudine and Combined Anti-tuberculous Agents in Rats

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Background: Advent of the HIV/AIDS pandemic has led to a dramatic increase in the number of TB cases worldwide. Availability of highly active antiretroviral therapy (HAART) has significantly improved the outcome of HIV/AIDS, in terms of prevention of opportunistic infections (OIs) as well as mortality however; liver toxicity is one of the most relevant adverse effects of antiretroviral therapy (ART).
Purpose: In view of the inevitable use of zidovudine (a common ART) and antituberculous fixed-dose combination therapy (FDCs) in the management of HIV-TB co-infection and the resultant hepatotoxicity, this study was aimed to investigate the hepatoprotective role of neutrosec (a combination of aminoacid and vitamins) on the hepatotoxicity induced by co-administration of zidovudine and combined fixed dose antituberculous agents.
Method: Twenty four rats were randomly allotted to four groups, consisting of the control, zidovudine plus fixed dose combined anti TB agents treated group, zidovudine plus fixed dose combined anti TB agents plus neutrosec treated group and neutrosec alone treated group. Therapeutic doses of zidovudine (8.5 mg/kg/day), fixed dose combined anti TB agents (25 mg/kg/day) and neutrosec (0.4 ml/kg/day) were administered to the animals via oral gavage, daily over 60 days. After 60 days, rats were sacrificed for internal macroscopic and histological examination of the liver. The liver enzyme parameters (AST, ALP, Total bilirubin, Total protein, Albumin) were determined using fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). Antioxidant enzymes activity and lipid peroxidation were determined according to standard procedures.
Results: The AST results showed a significant ($p \leq 0.05$) decreased in the zidovudine plus anti-TB plus neutrosec treated group (125.50 ± 22.71) compared with zidovudine plus anti-TB treated group (399.10 ± 0.45). It further showed non-significant decreased ($p \geq 0.05$) in the ALP levels between the zidovudine plus anti TB treated group (317.10 ± 73.48) and the zidovudine plus anti TB plus neutrosec treated group (203.20 ± 35.97). There was a non-significant ($p \geq 0.05$) decrease in the MDA level of the zidovudine plus anti-TB plus neutrosec treated group compared with the zidovudine plus anti-TB treated group.
Conclusion: The hepatotoxic effect of zidovudine plus combined anti TB drugs which may be due to free radical generation was modulated by neutrosec.

Key words: Fixed dose combined anti tuberculous agents, zidovudine, hepatic damage, oxidative stress, neutrosec

INTRODUCTION

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the Pharmaceutical industry and drug regulatory agencies [1]. According to the United States acute liver failure study group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%) [2]. Thus, drugs are important cause of liver injury and approximately 75% of the idiosyncratic drug reaction result in liver transplantation or death [3].

Isoniazid and rifampicin, which are some of the first line drugs used for tuberculosis therapy have been documented to be associated with hepatotoxicity [4, 5]. The rate of drug induced hepatotoxicity has been reported to be much higher in developing countries like India (8%–30%) compared to that in advanced countries (2%–3%) with a similar dose schedule [6]. The reported differences in the rate of hepatotoxicity may be due to incorporation of agents which may modulate the hepatotoxic effects of drugs in the developed countries.

Advent of the HIV/AIDS pandemic has led to a dramatic increase in the number of TB cases worldwide. Globally, 9 per cent of all new TB cases (31% in Africa) in adults were attributable to HIV/AIDS, as were 12 per cent of the 1.8 million deaths from TB, in the year 2000 [7]. World Health Organization (WHO) report estimated that in 2004, 8.9 million new cases of tuberculosis arose and 1.7 million deaths due to tuberculosis were recorded that year [8]. Over 80% of cases of TB/HIV co-infection were usually detected at early stages of HIV and an analysis of patients who died of AIDS revealed that 53% had tuberculosis [9]. According to the report of Raviglione [10]; TB is a leading cause of morbidity and mortality in patients

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with HIV/AIDS.

WHO predicts that a third of the HIV-positives will develop TB during their lifetime and gives several reasons for this co-infection. Some of these reasons are; HIV increases the risk of *M. tuberculosis* infection, HIV promotes progression to active tuberculosis in people with recently acquired and with latent *M. tuberculosis* infection, Increasing tuberculosis cases in people living with HIV possess an increased risk of tuberculosis transmission to the general public, and HIV increases the risk of recurrent tuberculosis [11].

Availability of highly active antiretroviral therapy (HAART) has significantly improved the outcome of HIV/AIDS, in terms of prevention of opportunistic infections (OIs) as well as mortality [12]. However, liver toxicity is one of the most relevant adverse effects of antiretroviral therapy (ART) [13-15]. There is no clear explanation of the mechanism of production of toxic hepatitis in patients undergoing ART therapy because; clinical presentation is the same as in other types of toxic hepatitis, although hypersensitivity reactions may appear later as therapy proceeds [16].

At present, there is no agreement on how to manage patients suffering from ART-associated liver toxicity. An algorithm has been proposed, which indicates different schedules of behavior depending on the presence of clinical symptoms [17]. According to this algorithm, it is recommended that ART be suspended in the case of grade 3 or 4 toxic hepatitis, regardless of the symptoms the patient presents [18]. Zidovudine (Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors) which is known to be a common drug in a typical 3-4 drug HAART regimen has been reported to be hepatotoxic [19-22].

Tuberculosis has been treated with combination therapy for over fifty years. Drugs are not used singly (except in latent TB or chemoprophylaxis), and regimens that use only single drugs result in the rapid development of resistance and treatment failure [23]. Recently, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) has recommended the replacement of single-drug preparations by fixed-dose combination therapy (FDCs) [24]. The justification for this recommendation is that FDCs provide a simple approach to delivering the correct number of drugs at the correct dosage as all the necessary drugs are combined in a single tablet [24, 25]. Combinations of two to four anti-TB drugs administered in fixed doses in a single tablet decrease the risk of developing multidrug-resistant tuberculosis [26]. Fixed-dose drug combinations have been found to be beneficial and cost-effective treatment for TB [27], however, with some systemic toxicities. Thus, the high incidence of anti-tuberculous drugs inducing hepatotoxicity may indicate difficulties with systematic steps in the prevention and management of anti-tuberculous drugs induced hepatotoxicity [6].

In view of the inevitable use of zidovudine (a common ART) and antituberculous fixed-dose combination therapy (FDCs) in the management of HIV-TB co-infection and the resultant hepatotoxicity, this study is aimed to investigate the hepatoprotective role of neutrosec (a combination of aminoacid and vitamins) on the hepatotoxicity induced by co-administration of zidovudine and combined fixed dose antituberculous agents.

METHODOLOGY

Drugs

Zidovudine USP 300 mg per tablet was obtained from President Emergency Programme for AIDS Relief (PEPFAR) centre in Lagos University Teaching Hospital, Lagos-Nigeria. Fixed dose combined antituberculous agents contain rifampicin 150 mg; isoniazid 75 mg; parazinamide 400 mg and ethambutol 275 mg per caplet. The fixed dose combination drugs were obtained from direct observed treatment (DOT) tuberculous clinic in Lagos University Teaching Hospital, Lagos-Nigeria. Neutrosec^R liquid (200 ml per amber bottle) contains methionine, choline and vitamins; it was obtained from a registered Pharmacy outlet in Lagos, Nigeria.

Animals

Rats were obtained from Laboratory Animal Centre of College of Medicine, University of Lagos, Nigeria. The animals were authenticated in Zoology department, Faculty of Science, University of Lagos, Nigeria. They were made to acclimatize for two weeks before the commencement of the experiment. The animals were fed on Pfizer Animal Feed cubes and water ad libitum. The investigation conforms to *The Guide for the Care and Use of Laboratory Animals* published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996)" for studies involving experimental animals.

Experimental Procedure

Twenty four rats (6 per group) were randomly allotted to four groups, consisting of the control, zidovudine plus fixed dose combined anti TB agents treated group, zidovudine plus fixed dose combined anti TB agents plus neutrosec treated group and neutrosec alone treated group.

Therapeutic doses of zidovudine (8.5 mg/kg/day), fixed dose combined anti TB agents (25 mg/kg/day) and neutrosec (0.4 ml/kg/day) were administered to the animals. The doses were administered via oral gavage, daily for 60 days. Rats in different groups were observed closely, looking for any behavioral changes, feeding and drinking habits, as well as body weight and general morphological changes. After 60 days, rats were sacrificed for internal macroscopic and histological examination of the liver was carried out. The liver enzyme parameters (AST, ALP, total bilirubin, total protein, albumin) were determined using fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany).

Measurement of both the serum and liver antioxidant enzymes activity and MDA levels were done according to standard procedures; catalase [28-30] and MDA [30, 31].

Results are presented as mean ± S. E. M. Statistical

Table 1   Determination of serum lipid peroxidation and anti-oxidant levels in the serum of treated rats

Groups (n = 6)	GSH (Umole/mg)	CAT (U/mg)	MDA (nmole/mg)
Control	0.55 ± 0.02	240.1 ± 36.93	0.04 ± 0.01
Zid + AntiTB	0.62 ± 0.13*	83.39 ± 14.02	0.09 ± 0.01
Zid + AntiTB + Nut	0.93 ± 0.19	120.10 ± 48.38	0.06 ± 0.01
Neutrosec alone	1.78 ± 0.38**	95.58 ± 19.77	0.12 ± 0.04

*$P \leq 0.05$ between Zidovudine +Anti-tuberculous (TB) agents group and Neutrosec alone group
**$P \leq 0.05$ Control group and Neutrosec alone group
MDA, malondialdehyde ; GSH, reduced glutathione, CAT, catalase

Table 2   Hepatic function parameters of treated rats

Groups (n = 6)	AST (U/L)	BIL (Mg/L)	ALB (g/L)	TP (mmol/L)	ALP (U/L)
Control	288.10 ± 36.22	0.85 ± 0.01	32.67 ± 0.84	70.67 ± 1.40	326.60 ± 8.36
Zid + AntiTB	399.10 ± 0.45*	0.84 ± 0.01	31.37 ± 2.12	63.13 ± 1.68	317.10 ± 73.48
Zid + AntiTB + Nut	125.50 ± 22.71**	0.83 ± 0.11	30.88 ± 1.19	69.66 ± 1.95	203.20 ± 35.97
Neutrosec alone	159.20 ± 38.58***	0.83 ± 0.01	34.48 ± 1.13	70.95 ± 4.46	257.30 ± 31.76

*$P \leq 0.05$ between Zidovudine + Antituberculous agents group and Zidovudine + Antituberculous+Neutrosec group
**$P \leq 0.05$ between Control and Zidovudine + Antituberculous + Neutrosec group
***$P \leq 0.05$ between Zidovudine + Antituberculous agents and Neutrosec group
Aspartate amino transferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (BIL), Albumin (ALB), Triglyceride (TP),

significance between the control groups and the test groups were analyzed by means of student t-test and ANOVA. *P* values less than 0.05 were considered significant.

RESULTS

Table 1 data showed the serum lipid peroxidation and anti-oxidants levels of rats treated with zidovudine plus combined anti-TB and zidovudine plus combined anti-TB plus neutrosec. The results showed significant ($p \leq 0.05$) increased in the level of GSH between control group (0.55 ± 0.02) and neutrosec alone (1.78 ± 0.38); also between zidovudine plus anti-TB treated group (0.62 ± 0.13) and neutrosec alone. There was slight non-significant ($p \geq 0.05$) increase in the level of GSH between zidovudine plus anti-TB group and zidovudine plus anti-TB plus neutrosec group (0.93 ± 0.19). There was a non-significant decrease ($p \geq 0.05$) in the catalase level between the control group (240.10 ± 36.93) and the zidovudine plus anti-TB group (83.39 ± 14.02). The catalase level was slightly increased in the zidovudine plus anti-TB plus neutrosec group (120.10 ± 48.38) compared with the zidovudine plus anti-TB treated group. The lipid peroxidation results showed a slight non-significant increase in the MDA level of zidovudine plus anti-TB treated rats (0.09 ± 0.01) compared with the control group (0.04 ± 0.01). The results also revealed a non-significant decrease in the MDA level of the zidovudine plus anti-TB plus neutrosec treated group (0.06 ± 0.01) compared with the zidovudine plus anti-TB treated group.

There was an unexpected but non-significant increase in the MDA level of neutrosec treated group (0.12 ± 0.04) compared with all other groups.

The results on Table 2 showed the hepatic function parameters of rats treated with zidovudine plus combined anti-TB and zidovudine plus combined anti-TB plus neutrosec. The data showed a slight non-significant increase in the level of AST between control group (288.10 ± 36.22) and zidovudine plus anti-TB treated group (399. 10 ± 0.45) and a significant ($p \leq 0.05$) decreased in the zidovudine plus anti-TB plus neutrosec treated group (125.50 ± 22.71). The results further showed non-significant decreased ($p \geq 0.05$) in the ALP levels between the zidovudine plus anti TB treated group (317.10 ± 73.48) and the zidovudine plus anti TB plus neutrosec treated group (203.20 ± 35.97). There was non-significant decreased in the level of total protein between control group (70.67 ± 1.40) and zidovudine plus anti-TB treated group (63.13 ± 1.68) with subsequent slight increased in the zidovudine plus anti-TB plus neutrosec group (69.66 ± 1.95) and neutrosec treated group alone (70.95 ± 4.46).

The results in Fig. 1 showed decreased in the liver catalase level between the control and zidovudine plus anti-TB treated group. There was a slight increase in the zidovudine plus anti-TB plus neutrosec group compared with the zidovudine plus anti-TB group. A significant increase was observed with the neutrosec treated group compared with all other groups.

There was an increase in the level of liver MDA in the zidovudine plus anti-TB treated group compared with the control group (Fig. 2). The results also showed a decrease in the MDA level of zidovudine plus anti-TB plus neutrosec treated group. An unexpected increase in the level of MDA was observed in the neutrosec alone treated group.

DISCUSSION

The advent of the HIV/AIDS pandemic has led to a dramatic increase in the number of TB cases
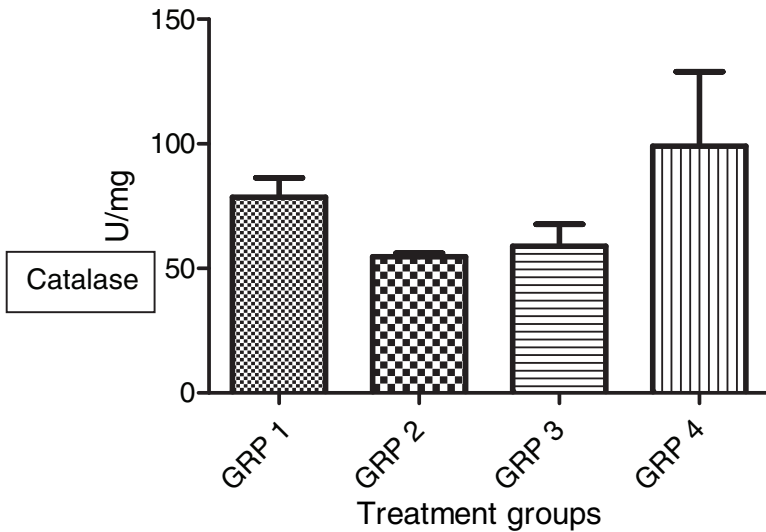
Fig. 1 Modulatory activity of Neutrosec on catalase level in the Liver of Zidovudine plus combined anti-TB treated rats.

Keys: Group 1, Control; Group 2, Zidovudine plus combined antituberculous agent; Group 3, Zidovudine plus combined antituberculous agent plus Neutrosec; Group 4, Neutrosec alone
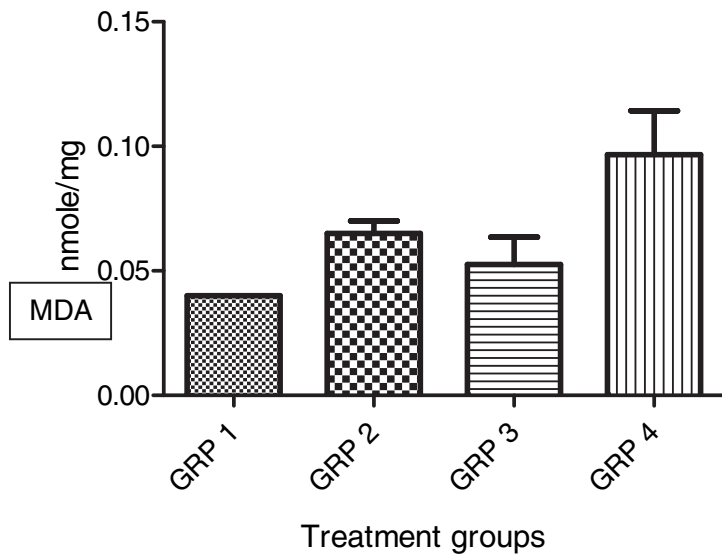
Fig. 2 Modulatory activity of Neutrosec on Lipid peroxidation (MDA) level in the Liver of Zidovudine plus combined anti-TB treated rats.

Keys: Group 1, Control; Group 2, Zidovudine plus combined antituberculous agent; Group 3, Zidovudine plus combined antituberculous agent plus Neutrosec; Group 4, Neutrosec alone

worldwide. Among opportunistic pathogens associated with the acquired immunodeficiency syndrome (AIDS), *Mycobacterium tuberculosis* is distinguished by its relative virulence and potential for person-to-person transmission [32, 33]. Persons infected with Human Immunodeficiency Virus (HIV) are particularly susceptible to tuberculosis, both from the reactivation of latent infection and from new infection with rapid progression to active disease [32, 33]. Globally, 9 per cent of all new TB cases (31% in Africa) in adults were attributable to HIV/AIDS [7]. Currently, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) has recommended the replacement of single-drug preparations by fixed-dose combination therapy (FDCs) [24] in the management of Tuberculosis. The justification for this recommendation is that FDCs provide a simple approach to delivering the correct number of drugs at the correct dosage as all the necessary drugs are combined in a single tablet [24, 25]. In the management of both tuberculosis and HIV/AIDS, drug-induced liver injury is a major health problem that challenges not only health care professionals but

also the pharmaceutical industry and drug regulatory agencies [1]. Zidovudine (Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors) which is known to be a common drug in a typical 3–4 drug HAART regimen has been reported to be hepatotoxic [19–22]. Also, rifampicin which is one of the major drugs in the fixed dose combination therapy (FDCs) has been evaluated to be hepatotoxic [5]. In an attempt to solve the problems of drug induced hepatic damage, this present study has investigated the possible roles of neutrosec (a combination of aminoacid and vitamins) in modulating the hepatic toxicity effects of FDCs and zidovudine.

The results obtained showed that combination of FDCs and zidovudine significantly ($p \leq 0.05$) increased the level of aspartate amino transferase (AST) in the treated rats. This combination also decreased the level of albumin and triglyceride compared with the control animals. However, the co-administration of neutrosec with combination of FDCs and zidovudine resulted in significant ($p \leq 0.05$) decreased in the level of aspartate amino transferase (AST) and marginal increase in the level of triglycerides. These observations may be
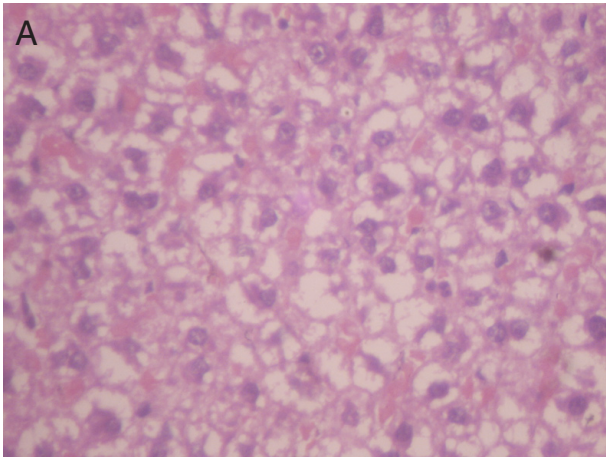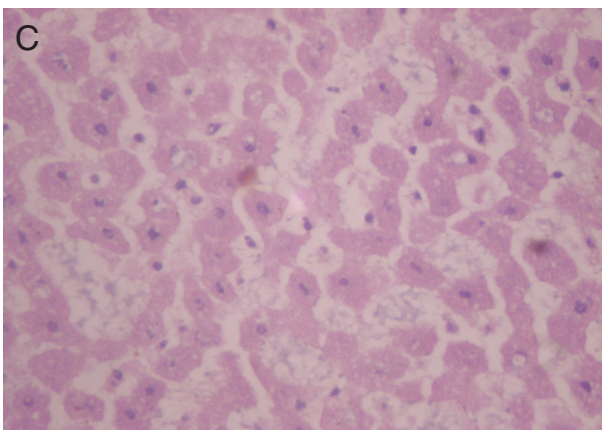
Fig. 3A Liver section of control rats
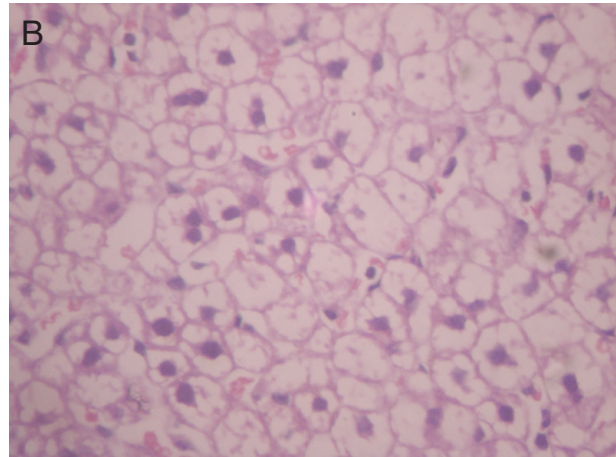
Fig. 3B Liver section of rats treated with Zidovudine + Fixed doseCombine anti TB (FDCs)

Fig. 3C Liver section of rats treated with combination of Zidovudine + Fixed doseCombine anti TB + Neutrosec

The histopathology results of the liver cells showed intracytoplasmic vacuoles and necrosis of focal areas of hepatocyte in the zidovudine plus FDCs treated group. There was no necrosis of the hepatocyte in the neutrosec plus zidovudine plus FDCs treated group (The magnification is x40).

due to combination of FDCs and zidovudine induced free radical generation which will subsequently cause oxidative stress in the hepatic cells leading to hepatic damage as seen in the data obtained from this study. The study revealed an increase in the level of lipid peroxidation of rats treated with combination of FDCs and zidovudine in both the liver and serum. However, combination of neutrosec with this combination of FDCs and zidovudine reduced the level of lipid peroxidation. More so, there was a decrease in both the levels of liver and serum catalase of animals treated with combination of FDCs and zidovudine but the co-administration of neutrosec with the combination of FDCs and zidovudine increased the levels of catalase in both the liver and the serum. The histopathology results of the liver revealed intracytoplasmic vacuoles and necrosis of focal areas of hepatocyte in the zidovudine plus FDCs treated group. However, no necrosis of the hepatocyte was seen in the neutrosec plus zidovudine plus FDCs treated group.

CONCLUSION

It is quite clear from the data obtained from this study that neutrosec which is a combination of amino acid and vitamins is able to positively modulate the hepatotoxic effect of combination of FDCs and zidovu-dine possibly by anti-oxidation (free radical scaveng-ing) mechanism. Thus, it may be advisable to always incorporate neutrosec in the regimen of HIV-TB co-infected patients as this may reduce the potential hepa-totoxic effect of this regimen.

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