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Full Length Research Paper

Effect of combination therapy of haart and α-zam herbal preparation for HIV infection in rats

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It is not uncommon seeing patients taking orthodox and herbal remedies concurrently for the same disease. The concern of many health practitioners is the safety of such combination therapy especially when there is a possibility of adverse drug reaction as a result of drug-drug interaction. This study focussed on drug interactions of α-zam (herbal antiretroviral drug) when combined with lamivudine, zidovudine and nevirapine (highly active anti-retroviral therapy) using an animal model. Wistar rats were divided into 5 groups (10 rats per group) and were administered with 400 mg/kg (α-zam), 800 mg/kg (α-zam), 400 mg/kg (α-zam) +HAART, 800 mg/kg (α-zam) +HAART and normal saline. All administration was done once daily orally via canula to the 4 different groups respectively with the 5th group as control for 6 days. All wistar rats in all groups were observed daily for change in behaviour. On the 7th day, all rats were bled via retro-orbital plexus under light diethyl ether anaesthesia. The blood samples were analysed for haematological (haemoglobin concentration, white blood cell, red blood cell, platelet and lymphocyte counts) and biochemical (electrolytes, urea, creatinine, lipid profile, liver and renal functions tests) parameters. Some organs were also harvested for histological examinations. There was neither mortality nor behavioural abnormality observed in all the drug administered rats. There were some mild atrophic changes in liver and kidney with hyper-cellularity of bone marrow but no statistically significant difference (P>0.05) were found in all blood (haematological and biochemical) parameters in rat groups administered combination therapy (herbal concoction+ HAART). It was concluded that the combined administration of α-zam and HAART (lamivudine, zidovudine and nevirapine) did not cause any significant adverse drug reaction. Rather beneficial effects were observed in amelioration of side-effects associated with the herbal concoction and orthodox anti-viral medicines.

Key words: Combination therapy, HAART, α-zam herbal remedy.

INTRODUCTION

Nigeria is the most populous country in Africa and it is estimated that about 5 millions of the population are infected with human immunodeficiency virus (HIV). Thus Nigeria has the third largest population in the World infected with dreadful virus (UNAIDS, 2006). Nigerians like to the use of herbal remedies for major illnesses. HIV infection has no cure medically serves as a catalyst to source for cure in herbal remedies (Elujoba, 2005). Since confirmation of the HIV infection in Nigeria in 1987 after identification of the virus in 1980's in America, Germany

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and France, herbal therapists in Nigeria have been searching for the cure (Abalaka, 2004; Barre-Sinoussi et al., 1983; Gallo et al., 1983). This led to many claimed curative medicines or vaccines emanating from Nigeria (Abalaka, 2004).

The safety of herbal remedies had been a major concern to many people especially when the chemical constituents of the product are not known. Herbal remedies are herbs, herbal materials, herbal preparations and finished herbal products, used to treat a multitude of ailments throughout the world (WHO, 2002). There are many classes of herbal remedies used for HIV infection based on its chemical constituents such as: alkaloids, carbohydrates, coumarins, flavonoids, lignans, phenolic, proteins, quinones, terpenes and tannins. One herbal remedy differs from the others in its therapeutic effects and toxicity depending on the chemical constituents (Cos et al., 2008).

There are many herbal remedies that are being used in Nigeria for HIV infection. Many of these herbal remedies are used as complementary therapy to HAART. Toxicological studies have been done on some herbal products in Nigeria using animal models (Abere and Agoreyo, 2006). Unlike the assumptions that herbal remedies are harmless because of the natural source, many have been found to be toxic (Keay et al., 1964). Thus safe herbal remedies are being identified and its use is encouraged while the use of harmful herbal products are discouraged (Sofowora, 1993). Unfortunately, many consumers did not know which herbal remedies are safe thus, general acceptance or rejection of the herbal products (Adefemi et al., 1988).

It was estimated that over 70% of HIV patients taking herbal remedies denied taking them when asked by medical practitioners (Dwyer et al., 1995) . This denial by HIV patients may constitute a deterrent to the medical practitioners in early detection of possible negative drug interactions that could occur with orthodox medicines especially HAART. It had been established that there was negative drug interactions between some herbs like garlic and St John's Wort with highly active anti-retroviral therapy (Nyika, 2007).

African potato (Hypoxis) and Sutherlandia frutescens have caused potential harmful interactions with anti-retroviral drugs (Mills et al., 2005). Even, the vitamins and cannabis had been documented to interact with orthodox medicines used for HIV infection (Dhalla et al., 2006). While some herbal remedies negatively interacted with orthodox anti-retroviral drugs, Coumarins decreased drug resistance resulting from HIV mutation associated with non nucleoside analogue-nevirapine (Dharmaratne et al., 2002; Yu et al., 2003).

Thus, herbs can be beneficial or harmful when used as complementary therapy. This led to investigating the adverse drug interaction of α-zam , an herbal remedy used as alternative therapy or complementary to highly active anti-retroviral therapy (HAART) in many parts of Nigeria (Onifade et al., 2011). α-zam is an herbal concoction that contained many plants derived chemical substances used for treatment of HIV infection (Onifade et al., 2010).

MATERIALS AND METHODS

Materials

α-zam

This is an herbal concoction that contained black seed (nigella sativa), honey and water as the major constituents as disclosed by the herbal therapist. - Zam phytochemistry revealed the presence of alkaloids, saponins, tannins, cardenolides and anthraquinones (Onifade et al., 2010).

Highly active anti-retroviral therapy (HAART)

The drugs used in this study were Nevirapine (50mg/kg), Lamivudine (100mg/kg) and Zidovudine (300mg/kg) prepared by grinding the tablets into fine powder.

Drug preparation

The herbal preparation was made using tepid distilled water as recommended by herbal therapist. The graded concentrations of 400 and 800mg/kg were prepared from 1g of α-zam concoction dispensed to HIV patients in paste form. The Nevirapine (50mg/kg), Lamivudine (100mg/kg) and Zidovudine (300mg/kg) and α-zam herbal concoction were mixed together before administered. Only fresh drugs (prepared daily) were used.

Animals

Wistar rats (150 to 200g body weight) were acclimatised for 7 days before the start of the experiment. Throughout the course of the experiment, they were housed under standard environmental conditions and maintained on a natural light and dark cycle. The animals had free access to rat chow and portable water.

Drug administration

The freshly prepared herb and HAART were administered orally using oral canula to animals once in 24 h. The herbal preparation and HAART (nevirapine, zidovudine and lamivudine) were administered concurrently to the rats (20 rats) receiving combination therapy. Animals were deprived of food before drug administration after which they were allowed access to food.

Experimental procedure

50 Albino rats were randomised divided into 5 groups (10 rats per group) and were administered once daily for 6 days with herbal concoction of 400, 800, and 400 mg/kg + HAART (Nevirapine, Zidovudine and Lamivudine), 800 mg/kg+ HAART (Nevirapine, Zidovudine and Lamivudine) respectively. The 5th group received normal saline and served as control. The feeding pattern and behaviour of rats were observed daily. 24 h after the last dose (the 7th day), the diethyl ether- anaesthetised animals were bled from the retro orbital plexus for haematological (total white blood cell count, red blood cell count, haemoglobin concentration, platelet count and lymphocyte counts) studies and serum biochemical

analysis (electrolytes, urea, creatinine, lipid profile, liver and renal functions tests) . The liver, kidney, spleen, skin and bone marrow were harvested for observation of any histological changes.

Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA). The significant difference ($P<0.05$) between drug administered and control groups was noted and indicated in the result.

RESULTS

The results of white blood cell count, red blood cell count, haemoglobin concentration, platelet and lymphocyte counts after administration of herbal preparation alone and combination of herbal preparation and HAART are shown in Table 1. The results of electrolytes and renal functions test (urea, creatinine) after administration of herbal preparation alone and combination of herbal preparation and HAART are shown in Table 2. The results of liver functions test and lipid profile after administration of herbal concoction alone and combination of herbal concoction with HAART are tabulated in Table 3. The histological changes after administration of herbal concoction alone and combination of herbal concoction and HAART are shown in Table 4.

DISCUSSION

The haematological parameters in Table 1 showed that -zam when used alone at concentration 400 mg/kg caused statistical significant ($P<0.05$) derangement in white blood cell count. The significant leucocytosis is of myeloid cells as evident with no significant difference in lymphocyte count. In addition, there was a significant leucocytosis (lymphocytes and myeloid cells) and thrombocytosis at 800 mg/kg when α-zam was used alone. However, neither 400 mg/kg and HAART nor 800 mg/kg and HAART caused leucocytosis or thrombo cytosis. Thus the bone marrow suppression potential (adverse effect) of lamivudine and zidovudine suppressed the α-zam bone marrow induction (hyper-cellularity). This haematological parameters finding is contrary to earlier study that herbal remedies could potentiate the toxicity of HAART (Mills et al., 2005).

The electrolyte, urea and creatinine of rats administered α-zam and HAART as shown in Table 2 showed that none of the electrolytes was significantly deranged. The renal functions were not impaired up to 800 mg/kg of –Zam concentration and HAART as complementary therapy or alone. The impairment of renal functions test which is a cardinal sign to nephrotoxicity was completely absent. This showed that there was no harmful drug reaction that could affect kidneys at concentration of 800 mg/kg of α-zam alone or as a complementary therapy with HAART. Thus the result of renal functions and electrolytes parameters is in supports of earlier study on safe combination of herbal remedy with orthodox medicines (Wang et al., 2010).

Liver is the primary organ involved in oral drug toxicity (Burtis et al., 2008). There was no significant difference in liver function tests parameters between the control group and rats administered with α-zam alone and combination with HAART group of animals in this study as shown in Table 3. The lipid profile results (Table 3) showed that there was an increase in high density lipoprotein (HDL) which is cardio-protective with a reduction in low density protein (LDL) in group of animals receiving combination therapy (400 mg/kg +HAART and 800 mg/kg+HAART). Thus the risk of atherosclerosis may be highly reduced in a combination therapy of α-zam and HAART. Saponin causes hyperlipidaemia and nevirapine induces hepatitis (Kindt et al., 2007). However, the combination of α-zam (contained saponins) and HAART (containing nevirapine) is found to be beneficial in this study.

The result of liver functions test and lipid profile corroborate earlier study that an herbal remedy prevented acetaminophen induced hepatitis (Wang et al., 2010).

The histological changes after administration of α-zam alone and in combination with HAART did not show significant derangement as seen in Table 4. Nyika (2007) reported that HIV patients on anti-retroviral drugs (HAART) taking St John's wort as complementary therapy induced irreversible injury in major organs in the body. However, in this study the complementary therapy of α-zam and HAART did not cause significant injury to the spleen compared with α-zam alone in the animal model. Likewise hypo-cellularity (bone marrow suppression) associated with Lamivudine and Zidovudine appeared to be over- shadowed with the effect of α-zam bone marrow induced hyper-cellularity.

The atrophic changes seen in liver and kidney in combination therapy of α-zam was mild (adaptational changes) and was not different from α-zam alone thus in contrary to earlier study that some herbal remedies are toxic and could cause severe harmful effect when used with orthodox drugs (Russo et al., 2009). Thus the adaptational histological changes in bone marrow, liver and kidney with no significant changes in skin and spleen when α-zam and HAART were used as combination therapy in this study is in keeping with the earlier study on nature generosity. Nevirapine is associated with severe liver injury (hepatitis) while Zidovudine and Lamivudine had been linked with severe bone marrow suppression (Kindt et al., 2007). α-zam constituents like alkaloid, saponins and anthraquinones are potential cytotoxic agents (Adebayo and Adegoke, 2008; Bringmann et al., 1999; Cos et al., 2008).

Contrary to the expectation of acute death or irreversible severe major organ damage manifesting as change in behaviour, it was observed in this study that none of the wistar rats died or changed in pattern of feeding or behaviour. Thus the potential adverse drug

Table 1. Haematological parameters of rats administered α-zam alone and combination therapy with HAART (values ± standard deviations).

Substance administered	White blood cell count (cells/mm^3)	Red blood cell count (x 10^6)	Haemoglobin (g/dl)	Platelet count (x10^5)	Lymphocyte count %	Total lymphocyte count
CONTROL	6400 ± 334	7.55 ± 1.2	13.4 ± 2.1	3.81 ± 1.9	68±6	4352 ± 1240
400 mg/kg(α-zam)	9600±228*	8.34±0.7	15.3±1.8	7.19±2.56	84±9	8064±2365
800 mg/kg(α-zam)	9400±887*	7.88±0.56	14.3±1.2	9.65±2.85*	85.6±8	8084±1890*
400 mg/kg(α-zam)+ HAART	7500±630	7.84±0.71	14.0±1.1	6.70±1.8	69±4.1	5175±234
800 mg/kg(α-zam)+ HAART	7200±420	7.44±0.80	13.8±1.9	4.87±1.2	68±2.9	4896±346

*-statistically significant (P<0.05)

Table 2. Electrolyte, Urea and Creatinine of rats administered after administration of α-zam alone or combination therapy with HAART (values ± standard deviations).

Substance administered	Na+(mmol/L)	K+ (mmol/L)	HCO3-(mmol/L)	Cl- (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
CONTROL	142±2.0	4.5±0.31	30±2.2	103±2.4	6.7±0.54	62±2.1
400 mg/kg(α-zam)	140±2.1	4.6±0.22	30±2.1	104±2.3	6.5±0.46	64±2.5
800 mg/kg(α-zam)	144±2.7	4.4±0.27	30±2.0	105±1.9	6.6±0.32	66±1.9
400 mg/kg(α-zam)+ HAART	140±2.2	4.6±0.14	30±1.4	104±2.1	6.6±0.5	63±2.3
800 mg/kg(α-zam) +HAART	142±2.1	4.5±0.26	30±0.9	103±2.2	6.7±0.5	62±1.9

Na+ - Sodium; K+- Potassium; HCO3- Bicarcobonate; Cl- Chloride

Table 3. Liver functions test and lipid profile after using Zam-z alone and combination therapy with HAART (values ± standard deviations).

Substance administered	Albumin (g/dl)	Globulins (g/dl)	AST (mmol/L)	ALT (mmol/L)	Total protein (g/dl)	HDL (mmol/L)	Triglyceride (mmol/L)	LDL (mmol/L)	Total cholesterol (mmol/L)
CONTROL	31±5	52±5	51±7	11±2	83±13	1.1±0.2	0.8±0.3	0.4±0.2	1.8±0.13
400 mg/kg(α-zam)	35±4	57±4	45±7.8	9±2.1	92±4.9	1.2±0.4	0.8±0.3	0.4±0.2	1.9±0.23
800 mg/kg(α-zam)	34±4.4	59±4	49±6.9	12±2.3	93±2.7	1.3±0.3	0.7±0.29	0.4±0.1	1.8±0.19
400 mg/kg(α-zam)+ HAART	34±2.3	55±3	48±4	11±0.5	89±5	1.4±0.2	0.6±0.21	0.2±0.1	1.7±0.24
800mg/kg(α-zam)+ HAART	35±2.2	55±3	50±4	10±1.4	90±6	1.5±0.2	0.6±0.2	0.1±0.1	1.8±0.23

ALT- Alanine transferase; AST- Aspartate transferase; HDL- High density lipoprotein; LDL- Low density lipoprotein

reactions of each component as complementary therapy (nevirapine, zidovudine, lamivudine and α-zam) neither caused death nor any change in behaviour or pattern of feeding of the wistar rats in this study.

Conclusion

It was concluded that combined administration of α-zam and HAART (lamivudine, zidovudine and nevirapine) did not cause any significant adverse drug reaction in the animal model. Rather beneficial effects were observed in amelioration of side-effects associated with the herbal concoction and orthodox anti-viral medicines.

REFERENCES

Abalaka JOA (2004). Attempts to cure and prevent HIV/AIDS in central Nigeria between 1997 and 2002: opening a way to a vaccine-based solution to the problem? Vaccine, 22(29-30): 3819–3828.

Abere TA, Agoreyo FO (2006).Antimicrobial and toxicological evaluation of the leaves of Baissea axillaries Hua used in the management of HIV/AIDS. BMC Complement Alter Med. 21(6):22.

Adefemi OA, Elujoba AA, Odesanmi WO (1988). Evaluation of the toxicity potential of Cassia podocarpa with reference to official Senna. West Afr. J. Pharmacol. Drug Res., 8(1): 41-48.

Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest C, Danguest C, Axler-blin C, Vezinet-Brun F, Ronzioux C, Rozenbaum W, Montagnier L (1983). Isolation of a T lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science, 220: 868-871.

Bringmann G, Wenzel M, Ross Kelly T, Boyd MR, Gulakowski RJ, Kaminsky R (1999). Octadehydromichellamine, a structural analog of the anti-HIV michellamines without centrochirality, Tetrahedron, 55: 1731–1740.

Burtis CA, Ashwood ER, Bruns DE (2008). Tietz fundamentals of clinical chemistry, 6th edition, (Saunders publishers) Missouri, USA. ISBN-978-0-7216-3865-2, pp. 363-696.

Cos P, Maes L, Vlietinck A, Luc Pieters L (2008). Plant-Derived Leading Compounds for Chemotherapy of Human Immunodefiency Virus (HIV) Infection –An Update (1998 – 2007). Planta Med., 74: 1323–1337.

Dhalla S, Chan KJ, Montaner JS, Hogg RS (2006). Complementary and alternative medicine use in British Columbia – a survey of HIV positive people on antiretroviral therapy. Complement Ther. Clin. Pract., 12(4): 242-248.

Dharmaratne HRW, Tan GT, Marasinghe GPK, Pezzuto JM (2002). Inhibition of HIV-1 reverse transcriptase and HIV-1 replication by Calophyllum coumarins and xanthones, Planta Med., 68: 86–87.

Dwyer JT, Salvato-Schille AM, Coulston A, Casey VA, Cooper WC, Selles WD (1995). The use of unconventional remedies among HIVpositive men living in California. J. Assoc. Nurses AIDS Care, 6: 17-28.

Elujoba AA (2005). Medicinal plants and herbal medicines in the management of opportunistic infections in people living with HIV/AIDS, Our experience so far**.** Being a Guest lecture presented at the National Scientific Conference organized by the Nigerian Society of Pharmacognosy (NSP) at Zaria, Nigeria, pp. 11-12.

Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS, Mann D, Sidhu GD, Stahl RE, Zolla-Pazner S, Leibowitch J, Popovic M (1983). Isolation of human T- cell leukaemia virus in acquired immune deficiency syndrome (AIDS).Science, 220: 865-867.

Keay RNJ, Onochie CFA, Standfield DP (1964). Nigerian Trees by Federal Department of Forest Research in Nigeria**.** Offset Lithography of the University Press, Nigeria, pp. 18-19: 65–67.

Kindt TJ, Goldsby RA, Osborne BA (2007). Kuby Immunology, Freeman and Company, New York, 6th edition, ISBN 13: 978-1-4292-0211-4. pp. 517.

Mills E, Foster BC, van Heeswijk R, Phillips E, Wilson K, Leonard B, Kosuge K, Kanfer I (2005). Impact of African herbal medicines on antiretroviral metabolism. AIDS 3; 19(1): 95-97.

Nyika A (2007). Ethical and regulatory issues surrounding African traditional medicine in the context of HIV/AIDS. Dev. World Bioeth., 7(1): 25-34.

Onifade AA, Jewell AP, Okesina AB, Ojezele M, Nwanze JC, Saka GO, Yong K, Adejumo BI, Igbe AP, Egunjoobi AO(2010). The Phytochemistry and Safety profile of α-zam , herbal remedy used for treatment of HIV infection in Nigeria. Trop J Health Sci. TJHS 2010-021(In press).

Onifade AA, Jewell AP, Okesina AB (2011). Virologic and Immunologic outcome of treatment of HIV infection with a herbal concoction α-zam , among clients seeking herbal remedy in Nigeria. Afr. J. Tradit Complement Altern. Med., 8(1): 37-44.

Russo R, Autore G, Severino L (2009). Pharmaco-toxicological aspects of herbal drugs used in domestic animals. Nat Prod Commun. 4(12):1777-1784.

Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa (2nd Edition), Spectrum Books Limited, Ibadan,Nigeria, pp. 1-153.

UNAIDS/WHO (2006). AIDS epidemic update: December, pp. 1–90.

Wang AY, Lian LH, Jiang YZ, Wu YL, Nan JX (2010). Gentiana manshurica Kitagawa prevents acetaminophen-induced acute hepatic injury in mice via inhibiting JNK/ERK MAPK pathway. World J. Gastroenterol., 16 (3):384-391.

WHO (2002). Traditional Medicine; Growing Needs and Potential, WHO Policy Perspectives on Medicines. World Health Organization, Geneva, pp. 1–6.

Yu D, Suzuki M, Xie L, Morris-Natschke SL, Lee KH (2003). Recent progress in the development of coumarin derivatives as potent anti-HIV agents. Med. Res. Rev., 23: 322–245.