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Research

Testicular Microstructure and Hormonal Profilae Following HAART Administration: The Role of Jatropha Tanjorensis

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ABSTRACT

Africa is endemic to the human immunodeficiency virus, acquired immunodeficiency syndrome(HIV/AIDS). In rural settings, the use of orthodox medicine with herbal medication is common. The efficacy of highly active antiretroviral therapy (HAART) has led to increased demand for its therapeutic use although reported to induce structural abnormalities in testicular tissues among other complications. *Jatropha tanjorensis* (JT) leaf has been reportedly for its numerous benefits which include; antioxidant, anti-anemic and spermatogenic potentials. This study investigated the alterations in testosterone and testis microstructure following the administration of HAART (Zidovudine, Lamivudine and Levirapine [ZLN]), JT ethanolic leaf extract and the concomitant administration of ZLN and JT using *in vivo* model. Twenty male Wistar rats weighing 163-267 g were allotted into 4 groups of 5 rats per group. Animals were fed with growers' pellet and provided water ad libitum. Group 1 served received placebo; group 2 received JT 670.8 mg; group 3 received ZLN 9.285 mg while group 4 received a combination of JT 670.8 mg and ZLN 9.285 mg per kg body weight respectively. All administrations were performed *via* oro-gavage for 7 days. On day 8, animals were humanely sacrificed under chloroform inhalation; with blood obtained for hormonal assay while the testes were harvested, weighed and processed for routine histology. Testicular histo-architecture revealed mild distortions in the seminiferous tubules of test groups, with significantly ($p<0.05$) increased testosterone compared to control. In conclusion concomitant administration of ZLN and JT possess testiculo-toxic potential.

Keywords: Herbal medicine, HAART, Jatropha tanjorensis, Testis, Testosterone.

INTRODUCTION

Plants have served as food and drugs to man, and the scientific exploration into plant derived benefits has assumed various dimensions particularly in the field of medicine, applied sciences and agriculture [1-3]. For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicines [4,5].

Jatropha tanjorensis (JT) is a common field crop in the rain forest zones of West Africa [6] JT belongs to the family "Euphorbiaceae" [7,8]. It is commonly called "hospital too far" or "catholic vegetable" in Nigeria [9] Some parts of Nigeria consume the leaves of JT as vegetables added to daily meals and also in southern Nigeria, the leaves have been used in the treatment of diabetes mellitus as it is said to possess anti-hyperglyceamic effects [10,11]. Phytochemical screening of JT leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins [12-15].

People living with HIV/AIDS (PLWHAs) are placed on management with highly active antiretroviral therapy (HAART), which is existing pandemic drugs, which are cytotoxic, and the most common toxicity associated with HAART drugs is hepatotoxicity, which is usually due to damage to mitochondria [16-18]. One of these drugs is the combination of Zidovudine, Lamivudine and Nevirapine. Zidovudine (ZDV) also referred to as Azidothymidine (AZT) was the first approved treatment for HIV, under the names Retrovir and Retrovis [19]. AZT use was a crucial breakthrough in AIDS therapy in the 1990s that significantly altered the course of the disease and helped to destroy the notion that HIV/AIDS was a death sentence [19]. Zidovudine is nucleoside analog nucleotide reverse transcriptase inhibitors (NRTI). Lamivudine is commonly called 3 TC and can also be used in the treatment of chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2 [19]. Nevirapine is a dipyridozepinone non-nucleoside reverse transcriptase inhibitor approved for use in HIV-infected patients, and its efficacy is well demonstrated in numerous clinical trials. It has activity against HIV-1 but does not have significant activity against HIV-2 or other retroviruses [20].

The testes (testicles) are the male gonad, a paired ovoid reproductive gland that produces sperms (spermatozoa) and the male hormones, primarily testosterone, and are suspended in the scrotum by the spermatic cord [21]. Overall testicular function is controlled by two independent mechanisms; the first is the biosynthesis of androgens by Leydig cells, and the second is the production of spermatozoa in the epithelium of seminiferous tubules. The main role of Leydig cells is the production of androgens, which control male libido and spermatogenesis [22].

The leaf extract of JT has hypoglycemic and antioxidant properties that make it a popular remedy for the treatment of diabetics, malaria and hypertension in Nigeria [10,11]. It was reported that

administration of JT leaf powder to rabbits resulted in improvement in hematological indices which revealed an enhancement of bone marrow function [11,14] reported that JT may have the potentials of enhancing spermatogenesis when consumed for a short period of time and that it was dose dependent [11]. However, JT like many members of the family Euphorbiaceae, contains several toxic compounds, including; lectin, saponin, carcinogenic phorbol, and a trypsin inhibitor. The plant also exhibits low hemaglutination properties indicating low toxicity on red blood cell. Studies have shown that the plant is no longer safe for use and that it could be toxic to organs in the body [23]. Yet this plant is still being consumed widely, therefore this study investigated the role of ethanolic leaf extract of JT following the administration of highly active antiretroviral therapy (LNZ) on the testosterone levels and histomorphology of the testes of adult Wistar rat.

MATERIALS AND METHODS

Animals handling and care

Twenty (20) male albino Wistar rats with weights ranging from 163-295 g were obtained from the Faculty of Science Animal House and kept in spacious wooden cages. The animals were assigned according to weight range into 4 groups, with 5 per cage to allow them a degree of freedom and they were given food and water ad libitum. The cages were cleaned and the beddings of wood shavings changed on daily basis under 12 hour light and dark cycle 6:30 am to 6:30 pm. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [24].

Collection and preparation of extract

The plant sample was collected in the month of June 2018 from the medicinal farm of the Department of Natural Medicine and Pharmacognosy, Faculty of Pharmacy, University of Uyo. It was identified by a taxonomist, in the Department of Botany and Ecological Studies, University of Uyo and the sample was deposited in the herbarium and the voucher name was (UUPH six).

The fresh leaves of the plant weighing 1 kg were pulverized and macerated in ethanol (99.7%) for 72 hours, and the mixture was stirred every 24 hours, after which it was filtered and the filtrate was concentrated to dryness in water bath at 45˚C. The concentrated yield of the extract 47.8 g was preserved in the refrigerator at -4˚C until the research commenced.

Determination of median lethal dose (LD50)

The LD50 was carried out using modified [25]. This method involves the administration of various dosages ranging from 100-5000 mg/kg body weight. Before administration, animals fasted for 18 hours (to avoid feed and drug interaction). LD50=√AB; where A=no mortality, and B=maximum mortality.

Experimental design

Twenty (20) animals were weighed and divided into four (4) groups; group 1 the normal control (NC) received placebo (distilled water) 5 ml/kg body weight, group 2 received 20% of the LD50 of JT ethanolic extract 670.8 mg, group 3 received HAART (ZLN) 9.285 mg, while group 4 received ZLN 9.285 mg and JT 670.8 mg per kg body weight respectively.

Drug acquisition

Highly active antiretroviral therapy (Zidovudine 300 mg, Lamivudine 150 mg and Nevirapine 200 mg [ZLN]) was sourced from the manufacturer Strides Acrolab Limited, Bangalore-562 106, India Mfg. Lic.No.:KTK/25/415/98).

***Weight and organo-somatic index determination*:** At the end of the administration of the drugs and extract, animals were weighed and used to calculate organo-somatic index by the formula: weight of organ/weight of animal x100.

Gross observation: Gross observation of the animals was done by daily examining the change in physical appearances of the animal's skin, eye and fur colour, and inflammation on the limbs and tail.

Histopathological assessment: The paraffin wax blocked of the testes tissues were sectioned at 5 microns using the rotary microtome, and stained with haematoxylin and eosin (H&E), and thereafter examined under light microscope (Olympus-CX31, Japan), with photomicrographs obtained with Amscope digital camera (MU1000 China).

RESULTS

The results and observations in this research work are grouped into phytochemical analysis, median lethal dose, body weights, organo-somatic indices, testosterone level and the histological findings.

Effect of HAART and Jatropha tanjorensis on testicular and body weights

There was no significant weight gain in group 1; however, group 2 demonstrated a decreased in the percentage change in body weight, while group 3 showed there was a net gain in the percentage change in body weight similarly in group 4. The organo-somatic indices were not statistically significant as shown in **Table 1.**

Effect of HAART and Jatropha tanjorensis on testosterone level

The results obtained for the testosterone level of the experimental Wistar rats are presented in **Table 2.** Serum testosterone levels significantly ($p<0.05$) increased in the test groups compared to the control.

Table 1: Effect of HAART and Jatropha tanjorensis on testicular and body weights.

Group	Initial body weight (g)	Final body weight (g)	% change in body weight	Organ weight (g)	Organo-somatic indices
NC	244.60 ± 13.20	244.60 ± 10.40	0	1.21 ± 0.08	0.49
JT	239.80 ± 3.31	220.75 ± 3.07	-7.94	1.24 ± 0.04	0.56
HAART	203.20 ± 1.59	213.00 ± 6.00	4.82	0.88 ± 0.26	0.41
HAART+JT	261.80 ± 1.62	275.00 ± 5.61	5.04	1.38 ± 0.08	0.5

Data is expressed as Mean ± SEM

DISCUSSION

The introduction of HAART in HIV/AIDS management has improved therapeutic results by reducing mortality rates. However, HAART has a number of adverse effects, probably due to associated drug-drug interactions or organ specific toxicity [26] had reported that HAART consisting of TDF, FTC and EFV has spermatotoxic effects which may be associated with the reduction in testicular antioxidant [26]. As such, JT which has been reported to be anti-anemic with antioxidant potential, was seen as a medicinal plant that can be utilized with HAART to reduce its histological and cytological effects [27,28].

The phytochemical analysis of JT revealed the presence of alkaloids, flavonoids, phenols, saponins and cardiacglycosides. These compounds are also known secondary plant metabolites and can be used as drug precursors and pharmacological analyte [19,29]. The presence of some of these compounds partially clarifies the reason it is used in traditional medicines for the treatment of ailments [29].

The body weight showed no significant increase in group 1, but group 2 showed a decrease in body weight while groups 3 and 4 showed a significant increase in body weight. The decrease in body weight with the administration of JT to an extent confirms the work of Iyare et al. Who documented that JT exerted weight loss at the dose of 250 and 500 mg/kg administered twice daily for 42 days [15]. Also, the increase in body weight with the administration of HAART (LNZ) is in agreement with the work of Oyeyipo and co-workers whose report showed an increase in body weight of experimental animals treated with HAART (TDF, FTC and EFV) [26].

Serum testosterone levels significantly increased in test groups as compared to NC group (8.58± 0.19). This increase may be due to a surge in the activity of the Leydig cells induced by the extract and HAART drug. This increase was however highly significant in the group concomitantly administered with HAART and JT, which may be as a result of drug-extract interaction on the Leydig cells, hence propelling testosterone production. The observed increase by HAART drugs confirms the findings of Dube and co-workers who reported that free testosterone increased significantly after initiating antiretroviral drugs [30] and as earlier affirmed that ART significantly increases testosterone level [31].

Histological photomicrograph of group 1 as shown in **Figure 1** showed a normal histoarchitecture of the spermatogenic germ cells in the seminiferous tubules, it served as control. Group 2 was administered with 670.8 mg/kg of JTE (20 % of JTE), the connective tissue of the seminiferous tubule appeared depleted; the spermatogenic cells appeared larger than normal and clustered together, the germinal cells appeared to have vacuoles and the spermatozoa appeared scanty compared to Group 1. It was observed that there was proliferation of cells at the basal region,

Table 2: Effect of HAART and Jatropha tanjorensis on testosterone level.

Group	Testosterone level (mg/ml)
NC	8.58 ± 0.19
JT (20% LD50)	9.15 ± 0.37*
HAART	9.15 ± 0.44*
HAART+JT (20% LD50)	11.15 ± 0.06*

*Significantly increased compared NC

Data is expressed as Mean ± SEM.

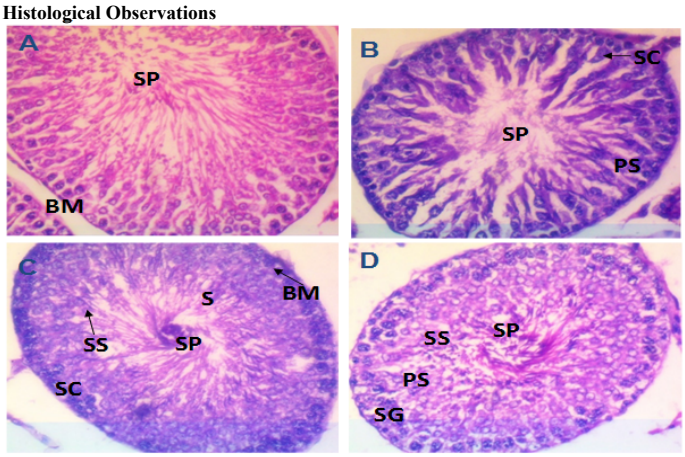

Figure 1: Effect of HAART and *Jatropha tanjorensis* on testis (H&E) x400.

leading to an increase in the number of spermatogenic cells from spermatogoonia pool to the spermatids. However, the scanty spermatozoa at the center may be as a result of a delay of the transformation of spermatid cells to form spermatozoa (a decrease in the rate of spermatogenesis). Moreover, it can be deduced that JT enhances the production of the spermatozoa at the level of the spermatogenic cells. Osuchukwu et al. had reported that JT may have the potentials of enhancing spermatogenesis when consumed for a short period of time. [11].

Group 3 treated with HAART of 9.285 mg/kg showed shrinkage of seminiferous tubule which caused the seminiferous tubules to appear smaller compared to the normal control group. Also, proliferation of the primary and secondary spermatocytes was observed. Some of the basal cells (spermatogonia) were darker and appeared to be necrotic. Inferentially, HAART 9.285 mg/kg enhanced spermatogenesis causing transformation of spermatids to spermatozoa, as the seminiferous tubules demonstrated congested lumen with dense presence of spermatozoa. However, the basal cells appeared to be affected and this necrotic activity may affect the spermatogenic cells if there is an increase in dosage and duration; and this will likely affect the function of the spermatozoa. This finding affirms the work of Chris-Ozoko et al. [19] who stated that Zidovudine may have a destructive effect on the histoarchitecture of the testes.

Group 4 treated with a combination of HAART 9.285 mg/kg and JTE of 670.8 mg/kg showed clustering of spermatozoa [32,33] scattered spermatogenic cells, larger basal cells with vacuolations. Thus, it can be said that the combination of both HAART and JT extract enhanced spermatogenesis but not without adverse effect to the spermatogonium, which corroborates histological features documented in [11] (whose histological section showed that irrespective of the filling of the lumen, there is mild to moderate distortion to the permatocytes).

CONCLUSION

In conclusion, this present study establishes that short term administration of Jatroipha tanjorensis extract at 670.8 mg/kg concomitantly with therapeutic dose of HAART Zidovudine-Lamivudine-Nervirapine at 9.9.285 mg/kg for one-week induced mild testicular toxicity to the spermatogenic lineage cells, but increased serum testosterone in Wistar rat.

AUTHORS'S CONTRIBUTIONS

E.I.B, I.A.E. and A.N.A. designed the study, analyzed/interpreted

data, and drafted the manuscript, while A.F.A. and I.A.E performed the experiments. All authors approved the final manuscript.

CONFLICT OF INTERESTS

Authors declare that there is none.

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