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Research Article

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Anti-Hepatitis B Viral Potentials of Ethanolic Stem Bark Extract of *Vitex doniana* and *Lophira alata* in Wistar Rats

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Abstract Anti-hepatitis B viral activity of ethanol stem bark extracts of *Vitex doniana* and L*ophira alata* in Wistar rats was investigated**.** Forty-two (42) humanized albino rats were used for the study. The animals were divided into six (6) groups of seven (7) animals each. Groups II-VI were inoculated with hepatitis-B virus at the concentration of 25µl of the virus preparation was inoculated into a day old suckling humanized Wistar rat via intra cerebral route using tuberculin syringe. Group 1 and 2 served as normal and positive controls respectively and were given Dimethylsulphoxide/distilled water as placebo. Group III received 300mg/kgBW of standard anti-retroviral drug (Tenofovir-Lamivudin), group IV received 244mg/kgBW of *Vitex doniana*, group V received 244mg/kgBW of *Lophira alata* and group VI received combination of *Vitex doniana* and *Lophira alata* both at 244mg/kgBW. The treatment was administered twice daily for ninety days with the aid of orogastric tube. Result of the study reveal a drastic reduction in viral load in all treated groups; groups III (172.47 ± 3.99), IV (18.58 ± 1.01), V (548.48 ± 3.26) and VI (0.00 ± 0.00) with more significant ($p<0.05$) reduction in the combined treated group; group VI (0.00 ± 0.00) which recorded no significant ($p<0.05$) differences and compared well with the normal control (0.00 ± 0.00). Results of biochemical indices determination revealed a significant ($p<0.05$) increase in AST activity in group VI compared with normal controls but was significantly lowered when compared with positive control. However, ALT activities in group V showed a significant ($p<0.05$) decrease while that of group VI was significantly ($p<0.05$) increased compared with normal controls. ALP activities showed significant ($p<0.05$) reduction in all treatment groups compared with the positive control. Total protein, globulin and albumin showed significant ($p<0.05$) increased in all treated groups compared with the positive controls. The results obtained for the above parameters in all treated groups may be as a result of the administration of the extracts which reduces the HBV viral load and hence, reversing hepatic necrosis and morphological distortion occasion by viral toxins on the liver tissues. Co-administration of the two plants extracts in the treatment of HBV-induced hepatitis in Wistar rats appears to be effective, safe at the dose investigated compared with the standard drug (Tenofovir-Lamivudin) and can be a candidate for further research and development new anti-viral drug.

Keywords Anti-hepatitis-B, *Vitex doniana, Lophira alata*, HBV induced hepatitis

Introduction

Plant materials are centrally used in ethnobotanical practices and have remained useful sources of new drugs [1]. Although orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world

[1,2]. In developing countries of the world, traditional herbal medicine is often used side by side with western medicine with herbal medicine taking the upper hand when western medicine is beyond reach [3].

In more recent years, with considerable research, it has been found that many plants indeed have medicinal values [4]. Plants known to be significant to biochemist and biomedical scientist have been components of phytomedicine since time immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents that can be derived from any part of plant such as; the bark, leaves, flowers, fruits, seeds etc *i.e*. any part of the plants may contain bioactive components [6]. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many scientific laboratories and analysis of plants components follows a logical path where plants are collected either randomly or by following leads supplied by local healers in geographical area where the plant are found in abundant [6]. *Vitex doniana* commonly called black plum is widely distributed in the eastern, north central and western parts of Nigeria with various parts of the plant used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, inflammatory diseases and viral infection etc [4].

As of 2006, there were some anti retro viral (ARV) medications/drugs licensed for the treatment/management of viral hepatitis infection globally. These include drugs like lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude) [7]. Ethno-pharmacological practitioners have made claims that so many plants parts including stem bark of *Vitex doniana* and *Lophira alata* has anti-hepatitis B properties/activities and so could be used in the management and treatment of viral induced hepatitis [8]. Despite this varied uses of plants, there are no sufficient scientific information on anti-hepatitis B viral activity of the leaves or other parts of *Vitex doniana* and *Lophira alata*. This study is therefore justify by the fact that it gives a scientific evaluation/investigation of the anti-hepatitis B viral activity of ethanolic stem bark extract of *Vitex doniana* and *Lophira alata* used in folk medicine by the people of Benue state, north central Nigeria and beyond in the management of hepatitis B viral infection.

Materials and Methods

Chemicals and Reagents

Assay kits used in this analysis were obtained from four separate laboratories: DIALAB Production and Vertrieb, RANDOX Laboratories limited, TECO Diagnostics, and Fortress Diagnostics Limited, supplied by Katchey Company Limited Nigeria.

Collection and Preliminary Processing of Plant Materials

The stem barks of *Lophira alata* and *Vitex doniana* were harvested from Mkar hill Gboko, Benue State, north central Nigeria. The authentication of the plants was carried by a botanist at Federal College of Forestry Jos Plateau State, with a voucher specimen number given as FHJO18 for *V. doniana* and FHJ032 for *L. alata* and the specimens were kept in the College herbarium.

The plants were thoroughly washed using running water and then distilled water was used to rinse. The barks of the stem were carefully peeled using a kitchen knife. The peeled stem bark were chopped into tiny pieces and dried at room temperature.

Preparation of Extracts

The dried barks were blended into powder using an electronic blender. The powder was weighed using an electronic weighing balance and were soaked in 80 % ethanol. The mixtures were then agitated using an electronic blender and were kept under a regulated temperature of 0-8 ºC for 48 hours (2 days). The extracts were doubly filtered using a Cheese cloth and then a filter paper. The filtrate was concentrated using a rotary evaporator at a regulated temperature of 37-45 ºC. The extracts were finally evaporated to dryness using an Electronic water bath.

Experimental Animals

Twenty-five mice (males and females) weighing between 25-30g and forty two albino rats weighing 80-120g were used for this study. Mice were gotten from the College of Sciences, Federal University of Agriculture, Makurdi,

Benue State while humanized albino rats were obtained from the animal facility National Institute for pharmaceutical research and development (NIPRD) Idu Industrial Layouta, Abuja-Nigeria. The mice were used for the determination and affirmation of Medial Lethal Dose (LD_{50}) while the rats were used for anti-viral screening. The animals were given one week acclimatization period, there after the animals were reweighed and housed in cages wire-mesh top and bottom, under monitored environmental conditions of temperature (28±2 ºC), relative humidity (50±5%) and a 12 hour light/dark cycle. The animal facility was properly ventilated and the animals where placed on the commercial rat pellet and water provided *ad libitum* during the experimental period.

Hepatitis B virus was titrated in one day old suckling Wistar rats as all Entero viruses are pathogenic to suckling rats and not adult ones. One box (Wistar Albino Rats) consisted of one mother rat and 6 baby rats which were used for each dilution. Concentration of 25µl of the virus preparation was inoculated into each pup by intra cerebral route using tuberculin syringe. Symptoms of pathogenic invasion were noticed by 48 hours; post inoculation deaths were recorded from day 3. Final reading was taken on the 5th day and the animals that survived the challenge and tested positive to the virus were used for the research.

Forty two animals were assigned into six (6) groups of seven animals each, used for normal and biochemical safety index analysis. Group 1-6 were treated according to the schedule in table 1. Treatments were administered twice daily *via* orogastric intubation for a period of ninety days (3 months).

Collection of Blood Samples for Analysis

The administration of the extract lasted for a period of ninety days after which the animals were sacrificed twelve hours after the last administration in accordance with the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes ETS-123 [9]. Whole blood was collected from the heart via cardiac puncture using sterile syringe and needle. The blood sample was put into plain sample tubes. Sera was obtained from the clotted blood in the plain sample tubes by allowing standing for 2 hours at room temperature to clot before centrifugation at 3000 rpm for 20 minutes using a bench top centrifuge, MSE England to separate cells from serum. Sera obtained from the respective samples were carefully removed using Pasteur pipettes and put into respective dry plastic specimen bottles that were labelled accordingly. These were kept frozen in a refrigerator while the viral load, HBsAg protein level and other biochemical parameters were estimated.

Biochemical Assays

Hepatitis B serum antigen level was determined b and hepatitis B viral DNA estimation was also carried out [10-12].

Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean and analyzed using the Analysis of Variance 'ANOVA; f-ratio' [13] and student 't' test where applicable. Values at $P < 0.05$ were regarded as significant in comparison with appropriate controls.

Result

The results of the assessment of anti-hepatitis B viral activity of ethanolic stem bark extracts of *Vitex doniana* and *Lophira alata* on humanized hepatitis B infected albino rats is presented in table 2 and 3 below. In this study, HBsAg and hepatitis B viral load levels were used to measure the efficacy/potency of these plants compared to commonly use (standard) anti-retroviral drugs.

Statistical evaluation reveals that the HBV DNA (iu/l) levels of animals in groups 3 (172.47 ± 3.99), 4 (18.58 ± 11.01) and 5 (548.48 ± 3.26) showed a significant ($p<0.05$) decreased when compared with the position control group (6114.96±5.50) but only animals in groups 4 (18.58 ± 11.01) and 6 (0.00 ± 0.00) recorded significant ($p<0.05$) decreased compared with the standard drug (53.2 ± 2.39) However, no significant ($p<0.05$) changes was observed when compared group 6 (0.00 ± 0.00) with normal control (0.00 ± 0.00). More so, similar trend was observed for HBsAg serum level at ($p<0.05$) significant level.

Results of biochemical investigation indicates that the AST activity (iu/l) of animals in groups 3 (77 ± 2.54), 4 (112.6 ± 2.07) and 5 (82.2 ± 6.97) showed a significant ($p<0.05$) increase when compared with standard drug (53.2

± 2.39) but no was significant ($p<0.05$) changes were observed when compared with control (72.8 ± 1.05). However, the AST activity for *Lophira alata* treated group 4 (112.6 ± 2.07) showed a significant increase compared with the control (72.8 ± 1.05) at ($p<0.05$) significant level. More so, result of ALT activity of animals in groups 3 (64.4 ±2.07) and 5 (58.2 ± 1.92) showed a significant ($p<0.05$) decreased when compared with that of group 2 (77.6 ± 2.30) and group 1 (71.2 ± 3.27). Besides, the ALT activity of animal in group 4 (106.8 ± 2.28) showed a significant ($p<0.05$) increased compared with standard drug (77.2 ± 2.30) and control (71.2 ± 3.27) at ($p<0.05$). However, ALP results obtained showed that the activity of ALP in group 3 (299.2 ± 2.59), 4 (64.4 ± 2.07) and 5 (324 ± 3.54) showed a significant ($p<0.05$) decrease compared with standard drug (371.2 ± 4.12) and when compared with the control (321.8 ± 4.12) showed no significant ($p<0.05$) increased.

The total protein result obtained indicated that the total protein level of animals in group 3 (64.8 ± 1.79g/l), 4 (65.80 ± 0.37g/l) and (56.4 ± 2.97g/l) showed no significant ($p<0.05$) changes when compared with standard drug (70.6 ± 2.19g/l) and the control (62.8 ± 2.59g/l) at ($p<0.05$) while investigation also reveals that the albumin levels of animals in group 3 (34.8 ± 1.92g/l) and 5 (31.8 ± 1.30g/l) showed no significant ($p<0.05$) changes when compared with standard drug (32.80 ± 2.92g/l) and control (31.8 ± 1.30g/l). However, the *Lophira alata* extracts treated group 4 (24.8 ± 0.84g/l) recorded a significant ($p<0.05$) decrease compared with both standard drug (32 ± 2.29g/l) and the control (31.2 ± 2.59g/l) at ($p<0.05$) significant level.

Serum globulin result also reveals that the serum globulin level of group 3 (31.6 ± 2.074) and group 5(25.8 ± 3.96) showed no significant ($p<0.05$) changes when compared with standard drug (37.60 ± 2.17) and the control (26.2 ± 1.92). However, group 4 (43.2 ± 2.18g/l) showed a significantly ($p<0.05$) higher level when compared to both standard drug (37 ± 2.17) and the control (26.20 ± 1.92g) at ($p<0.05$) significant level. Generally, the serum protein, albumin and globulin showed no significant changes except for the fluctuation in *Lophira alata* treated groups.

Discussion

The liver is the major site of biotransformation by which a harmful/toxic compound are transformed into less harmful form to reduce toxicity [14]. It is also prone to xenobiotic metabolism since its portal location is within the circulatory system [15]. The liver remains the target site of HBV (and other hepatitis viral) toxin and normally at a long run result in the distortion of morphological and structural integrity of the liver and thus disrupting the physio biochemical activities of the liver [16]. The extent of damage depends on the level of toxin released or produce by the virus visa via the level of the virus (HBV) in circulation [16].

The measurement of the levels of HB viral load and HBsAg level in body fluids can be used in assessing the degree of assault and the toxicity of the virus the liver [17-18] and such measurements can also be used to extrapolate the possible tissue cellular damage caused by the viral toxin long before it is revealed by histology.

In the present study wherein the anti-HBV activity of *V. doniana and L. alata* in stable HBV-transfected albino rats which can continuously produce high level of viral proteins was investigated. It was observed that the ethanolic stem bark extracts of *V. doniana and L. alata* decreases the secretion of HBsAg and the HBV viral load alongside the serum levels of HBsAg in all extracts treated groups but most especially in *V. doniana* and *L. alata* combined treated group. These results demonstrated for the first time that *V. doniana and L. alata* extracts might display inhibitory activity against HBV replication *in vivo*. Xu *et al.,* (2008) have similarly reported that green tea extracts inhibit HBV *in-vitro* [19].

The biochemical findings from this study showed that there were significant differences in value of serum AST, ALT and ALP, with the controls and the treated groups in the study. The significant change in the *Lophira alata* mentioned parameters are more likely to be due to the effect of the extract on the liver cells architecture, this is because the two markers of hepatic injury [20-22]. AST and ALT was significantly elevated while ALP was significantly decreased in extract treated groups. Significant high level of AST is common in most acute hepatocellular disorder with ALT being higher than or equal to AST. In addition prior studied have empirically show that these transaminases enzymes are widely distributed in other cells of the body and the serum level could be elevated in injury affecting these cells such as muscles injury due to strenuous exercise and myositis [23]. Nevertheless, the activities of ALT outside the liver cell are very low and therefore, ALT is considered more

specific for the hepatocellular damage than other enzymes [24]. The significant increased caused by the ethanolic stem bark of *Lophira alata* on serum globulin may indicate the potential of the plant extract to stimulate antibody production which is in agreement with [25] and this could account for it rampant usage in folklore medicine as anti-microbial agent. Albumin is the protein with higher concentration in the plasma but produced exclusively by the liver. It transports many small molecules in the blood and maintain colloid osmotic pressure. Since albumin production in group 4 decreases, it may indicate that the extract promotes poor functioning of the liver, the extract tends to be hepatotoxic or was unable to lower the viral load drastically.

Conclusions

Conclusively, that the ethanol stem bark extract of *V. doniana and L. alata* may be good candidate for further studies and drug development. There is no 100% antiviral therapies against HBV infection that is satisfactory been fully developed. Several pharmacological strategies are recently being implemented to manage infected persons. Thus, it is imperative that new and effective anti-HBV therapy be unveiled to manage patients as well as good numbers of carriers of chronic HBV since hepatomas can develop subsequently if not well managed.

Table 1: Experimental design and treatment schedule for anti-hepatitis B viral screening of the ethanol stem bark extracts of *Vitex doniana* and *Lophira alata* in Wistar rats

Group	Number of rats	Treatment
1 (Normal Control)	7	10% Dimethylsulphoxide
2 (Positive Control)	7	10% Dimethylsulphoxide
3 (300mg/kg)	7	Tenofovir-Lamivudine
4 (244mg/kg)	7	Stem extract of V*itex doniana*
5 (244mg/kg)	7	Stem extract of *Lophira alata*
6 (244mg/kg of each extract)	7	Stem extract of *Lophira alata* and *Vitex doniana*

Table 2: Effect of ethanolic stem bark extract of *Vitex doniana*, *Lophira alata* and antiretroviral drugs on Weekly HBsAg reactivity screening of Wistar rats

Group	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9	WK10	WK11	WK12
Group I (NC)	-	-	-	-	-	-	-	-	-	-	-	-
Group II (PC)	+	+	+	+	+	+	+	+	+	+	+	+
Group III (STD)	+	+	+	+	+	+	+	+	+	+	+	+
Group IV (V.d)	+	+	+	+	+	+	+	*	*	*	*	*
Group V (L.a)	+	+	+	+	+	+	+	+	+	+	+	+
Group VI (V.d & L.a)	+	+	+	+	+	+	*	*	*	-	-	-

Key: NC- Normal control, PC-Positive control, STD- Standard drug, V.d- *Vitex doniana,* L.a- *Lophira alata,*Vd- *Vitex doniana* and *Lophira alata*,. n=7, +-Reactive, *-faintly reactive, - =Not reactive, WK-Week

Table 3: Neutralizing activity of *Vitex doniana* and *Lophira alata* ethanol stem bark extracts against Hepatitis B virus in Wistar rats

Group	HBV DNA (iu/l)	HBsAg
Group I (NC)	0.00±0.00	0.00±0.00
Group II (PC)	6114.96±5.50	22.23±1.14
Group III (STD)	172.47±3.99	13.89±0.37
Group IV (V.d)	18.58±1.01	11.47±0.32
Group V (L.a)	548.48±3.26	16.13±0.45
Group VI (V.d & L.a)	0.00±0.00	0.40±0.09

Key: NC- Normal control, PC-Positive control, STD- Standard drug, V.d- *Vitex doniana,* L.a- *Lophira alata,*Vd- *Vitex doniana and Lophira alata*, HBsAg- Hepatitis B surface antigen, ND-Not detected. n=7

Table 4: The effect of ethanolic stem bark extracts of *Vitex doniana* and *Lophira alata* liver enzymes of Wistar rats

Group	**AST(iu/l)**	**ALT(iu/l)**	**ALP(iu/l)**
Group 1 (Control)	72.8±1.05	71.2±3.37	321.8±4.91
Group 2 (Tenofovir)	53.2±2.39	77.600±2.30	371.2±4.12
Group 3 (*Vitex doniana* extracts)	77±2.54[a]	64.4±2.07	299.2±2.59[a]
Group 4 (*Lophira alata* extracts)	112.6±2.07*[a]	106.8±2.28*[a]	324±3.54[a]
Group 5 (*Vitex doniana* and *Lophira alata*)	82.2±6.97[a]	58.2±1.92*	297.4±4.04[a]

Values expressed as mean ± SEM, n =7. *significant at P<0.05 compared with the Group 1 (Control) .[a]significant at P<0.05 compared with the Group 2 (Drug).

Table 5: Effect of *Vitex doniana* extracts, *Lophira alata* extracts and Tenofovir drug on protein concentrations of Wistar rats

Group	**TP (g/l)**	**Alb(g/l)**	**Glo(g/l)**
Group 1 (Control)	62.8±2.59	31.2 ±2.59	26.2±1.92
Group 2 (Tenofovir)	30±1.01		
Group 3 (*Vitex doniana* extracts)	70.6±2.19	32±0.32	37±2.17
Group 4 (*Lophira alata* extracts)	64.8±1.79	34.8±0.84	31.2±2.074
Group 5 (*Vitex doniana* and *Lophira alata*)	67.8±3.03	24.8±0.84*[a]	43.2±2.18*
Group 6 (*Vitex doniana* and *Lophira alata* extracts)	54.4±2.97	31.8±1.96	25.8±3.96

Values expressed as mean ± SEM, n =7.*significant at P<0.05 compared with the Control [a]significant at P<0.05 compared with Group 2 (Drug).

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