Hepatotoxicity and Nephrotoxicity Studies of Ethanol Extracts of *Annona Muricata* Leaves and *Fagara Zanthoxyloide* Roots on Zidovudine-Induced Wistar Rats


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The study determined the effects of administration of ethanol extracts of *Annona muricata* leaves (AML) and *Fagara zanthoxyloide* roots (FZR) on liver and kidney indices in zidovudine (ZDV)-induced Wistar rats. Animals were grouped into five (5); group 1 served as normal control, groups 2-5 were induced with 100g/ml ZDV/Kgbw and group 3 treated with 35IU/Kgbw of erythropoietin, group 4 with 4.5g/ml AML/Kg bw and group 5 with 3.8g/ml FZR/Kg bw for six weeks. Serum liver enzymes, other biochemicals (total protein, albumin, globulin, bilirubin, urea and creatinine), organ weights and histological examination were used to assess the impact on the liver and kidney using standard methods. A significant elevation (p≤0.05) in the activities of serum liver enzymes and bilirubin accompanied by a significant decrease (p≤0.05) in the other biochemicals were observed in group 2. Treatment with extracts resulted in a modulation of the induced effects of ZDV causing a significant decrease in the serum liver enzymes, bilirubin and increase in the total protein, albumin and globulin concentrations. Histology of the liver showed altered architecture, blood vessel congestion and necrosis; while the kidneys were hypoplastic with signs of swelling (group 2) which was gradually reverted on treatment with extracts. The study suggests that extracts of AML and FZR may confer some degree of protection to the liver and kidney.

Keywords: *Annona muricata*, *Fagara zanthoxyloide*, zidovudine, hepatotoxicity, nephrotoxicity, Wistar rat

INTRODUCTION

Plants are fast becoming an important source of life-saving medications for individuals with several symptoms or ailments. The preference and increasing interest given to traditional system of medicine over synthetic/orthodox medication has spurred research into the toxicological impact of these plants with regards to hepatotoxicity and nephrotoxicity as these organs serve as initial contact points for chemicals and toxins as they enter and as they are excreted from the body. The liver as a vital organ, is involved in the metabolism of drugs and exogenous compounds, breakdown of nutrients, the buildup of body tissues, storage sites for vitamins and minerals and elimination of toxins (Adisa et al., 2014). The kidneys are involved in the removal of waste products, re-absorption of nutrients and maintaining electrolyte concentrations in the body etc. As these two organs are of great importance for biotransformation (liver) and the subsequent excretion (kidney) an impairment in their function would adversely affect their health status (Belonwu et al., 2013). The degree of impairment may be measured by the activities of several liver enzymes in serum dislodged as a result of damage to the liver or by a measure of the concentration of certain biochemical compounds (bilirubin, creatinine, urea) in the blood produced by or expected to be biotransformed by the liver and excreted by the kidney (Adisa et al., 2014).

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Annona muricata (AM) commonly called soursop in Nigeria is widely distributed in the tropical regions of West-Africa. The leaves, barks, fruits and seeds have been recorded to possess several medicinal applications (Badrie and Schauss, 2009). The leaves have been applied or ingested as decoctions in cases of mild pains and also used in the treatment of symptoms associated with cold and asthma (Coria-Tellez et al., 2018). The barks and seeds have been used in the treatment of hypertension, diabetes, bacterial infections, inflammatory diseases and cancer (Ezurike and Prieto, 2014; De souza et al., 2011). They are reported to be rich in alkaloids, phenols, essential oils, acetogenins, carbohydrates, fiber and other compounds (Ekere et al., 2019).

Fagara zanthoxyloide (FZ) is a plant common to the rain forests of Southern Nigeria. The leaves have been reported to contain alkaloids, steroids, lignans and carbohydrates (Ekere et al., 2019). The most common traditional application is in the treatment of toothaches, urinary and venereal disease. It has also been used as components of antiseptics, anti-parasitic, anti-hypertensive, anti-inflammatory and analgesics (Adelisoye et al., 2012; Ogwal-Okeng et al., 2003). The most important discovery on the roots of Fagara zanthoxyloide is their proposed use as an anti-sickling agent due to the activity of 2-hydroxymethyl benzoic acid (a bio-component of the root extract) on the red cell membrane and preservation of the colour of the blood.

Zidovudine is a nucleoside reverse transcriptase inhibitor initially applied in the treatment of cancer and currently being used as part of highly active antiretroviral therapy (Ekere and Ikpema, 2019; Peters et al., 2014). It has been linked with several side effects such as anaemia, loss of appetite, weight loss, leucopenia, oxidative stress, thrombocytopenia, nausea, vomiting and shortness of breath etc. The culmination of these effects and the important role this drug plays in the maintenance of health in conditions of immunodeficiency has spurred research into tackling the side effects of this drug.

The saying that health is wealth is one that has been said too many in Nigeria. The management of the health status in rural areas in Nigeria is plagued by the occurrence of several side effects which according to medical practitioners interviewed ranged from appetite loss, weight loss, immune suppression, anaemia etc. In lieu to curb mitigating side effect, several rural dwellers indulge in the consumption of several tradio-medical concoctions. Several plants are acclaimed to be wonder-working by traditional medicine dispensers in South-southern Nigeria and include Annona muricata and Fagara zanthoxyloide. This study aims at evaluating and providing insights into the long-term toxicological impacts of the extracts of these plants on the liver and kidney in cases of possible side effects induced by zidovudine in Wistar rats (Ekere and Ikpema, 2019; Ekere et al., 2019, Peters et al., 2014).

MATERIALS AND METHOD

Collection of plant materials

Annona muricata leaves (AML) and Fagara zanthoxyloide roots (FZR) were obtained from Alakahia, Obio/Akpor L.G.A, Rivers State, Nigeria. The plant materials were identified by Dr. B. Chikezie of the Plant Science and Biotechnology Department, University of Port-Harcourt.

Preparation of plant materials

The AML and FZR collected were sterilized with a stream of distilled water and left to air dry at 25°C. They were pulverized with a mechanical grinder until a uniform coarse powder is obtained for each.

Extraction of plant materials

The dried powdered materials (300g) were placed in a conical flask and extracted using 3 litres of absolute ethanol for 1 week. The mixture was centrifuged and filtered. The residue was evaporated to dryness and stored at 4°C in a refrigerator.

Animal Experimental treatments

Seventy (70) albino rats weighing (200-250g) were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria and divided into five groups of 15 rats each; and housed in Griffin and George modular cage system

Group 1: Served as the normal control group which received only water and normal feed
Group 2: Served as the negative control group which received 100mg/ml of ZDV/Kg bw
Group 3: Served as the positive control which received 35IU EPO/Kg bw
Group 4: Received 100mg/ml ZDV/Kg bw and 4.5g/mlAML/Kg bw
Group 5: Received 100mg/ml ZDV/Kg bw and 3.8g/mlFZR/Kg bw

METHODS

Zidovudine (ZDV) was administered for two weeks in groups 2-5, prior to experimental treatment with extracts while treatment with extracts was performed for a period of 6 weeks. Twenty-four (24) hours after termination of experimentation, the animals were weighed and anaesthetized in chloroform. Blood samples were collected into heparinized bottles from the jugular vein and centrifuged at 500 rpm for 12 minutes to obtain the serum. The liver and kidney were also excised after dissection; with the variations in body and organ weights determined by the method described by Akinnawo et al., (2005).
Analysis of liver and kidney indices

Assay for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum were performed by the method described by Reitman and Frankel (1957) using Randox test kits. Serum urea concentrations was measured enzymatically with urease and glutamine dehydrogenase, creatinine levels were determined by the kinetic calorimetric method with alkaline picate and bilirubin concentration was performed by the methods described by Yakubu et al., (2007). Total protein, albumin and globulin were assayed according to the method described by George, (2009).

Histological examination

The excised organs were histologically examined by the method described by Al-Hasawi and Al-Harbi (2014).

STATISTICAL ANALYSIS

Values obtained were expressed as Mean ± Standard error of mean with analysis performed using SPSS software version 20 for Windows (SPSS Inc. USA) at p≤0.05 significant level.

RESULTS

Body and organ weight

The results of the variations in body and organ weights as presented in Figures 1-3, showed a decrease in the body weight of group 2 when compared to the normal control (group 1) but significant increases (p≤0.05) in the body weight in groups administered when compared to the negative control group. Treatment with extracts of AML resulted in an increase in the body weight within the group as time progress with the values observed to be significantly higher (p≤0.05) than the negative control group but lower in the normal control. The body weights on treatment with FZR were significantly higher (p≤0.05) also followed a similar trend in comparison with the AML and FZR treatment but the weights were significantly higher (p≤0.05) with FZR than AML.

The results of the body weight in group 2 may culminate the findings that the drug induces loss of appetite and may elicit certain factors which results in a subtle derangement of the neurochemical signaling with regards to appetite stimulation in the brain (Satya et al., 1999). The resultant effect would be a termination of neuromodulators related to appetite thus reducing food intake and concomitantly body weight loss. The results of group 4 and 5 may suggest that extract co-administration possibly through the presence of some bioactive compounds (Ekere et al., 2019), may have cushioned or reversed the neurochemical derangement thereby regulating food intake and thus body weight (Figure 1).

The weights of the liver and kidney were also observed to decrease in group 2 when compared to the normal control (group 1), while treatment with extracts of AML and FZR resulted in an increase in the liver and kidney weights.

Figure 1: Body weights in zidovudine induced rats treated with extract of Annona muricata leaves and Fagara zanthoxyloide roots.

Figure 2: Liver weights in zidovudine induced rats treated with extract of Annona muricata leaves and Fagara zanthoxyloide roots.

Figure 3: Kidney weights in zidovudine induced rats treated with extract of Annona muricata leaves and Fagara zanthoxyloide roots.

Organ weight, judged as a sensitive indicator of toxicity even in the absence of any morphological changes (Mandal et al., 2012) was observed to reduce in the negative control group when compared to the normal control; possibly due to the reduction in body weights observed during the study (Nirogi et al., 2013). The reductions observed at weeks 4 and 6 may be linked to an underlying pathological condition such as necrosis and degenerative disorders (Miyauchi et al., 2013). The increases in organ weight observed in treatment groups may signify proper metabolism or histological disorder.
(abnormal mass, contusions or tumours) which can be ruled out on histological examination (Miyauchi et al., 2013).

Liver and kidney indices

Results of the activities of liver enzymes are shown in Figure 4-6. There was significant increase in the AST, ALT and ALP activities after administration of 100mg ZDV/Kg bw (Group 2) when compared to the normal control (Group 1). The positive control (Group 3) also showed an increase in the serum activities of the liver function enzymes investigated when compared to the normal control (Group 1) but an insignificant reduction (p≤0.05) when compared with the negative control (group 2). Extracts of AML and FZR (Groups 4 and 5) resulted in significant reductions (p≤0.05) in the activities of the liver function enzymes at week 2 when compared with the negative control (group 2). Prolonged simultaneous administration (weeks 4 and 6) resulted in a reduction in the serum activities of AST and ALT but an increase in the ALP activity when compared with both negative and positive control groups.

The significant increase (p≤0.05) in the activities of liver enzymes in group 2 (Figure 4-6) may suggest hepatotoxicity which progressed to weeks 6 (Belonwu et al., 2013). Some researchers (Ogunka-Nnoka et al., 2019, Obi et al., 2014 and Imafidon and Okunrobo 2012) have reported that elevated levels of ALT, AST and ALP in serum are associated with acute liver toxicity. Adebayo et al., (2010) also reported that a deranged increase in ALP activity as observed in this study may be an indication of bone marrow disorder induced as a result of ZDV administration. Hence the changes in AST, ALT and ALP may suggest that ZDV induces hepatic damage and/or disorders which relate to/or are associated with osteotoxicity or myelotoxicity. The significant reductions in the activities of liver enzymes on administration with extracts as seen in groups 4-5 when compared with group 2 may also suggest the ability of these plants to confer protection to the liver against ZDV induced toxicity; confirming the beneficial effects of these plants in alcoholic hepatitis conditions as proposed by folkloric medicine dispensers (Adebayo et al., 2010). The decreases in the ALP activity may also suggest its use in conditions which results from bone marrow related toxicity.

The result of the administration of ZDV (group 2) on selected liver biochemical indices shown in Figure 7-10 revealed significant reductions (p≤0.05) in the concentrations of total protein, albumin and globulin when compared with the group 1. The treatment with AML and FZR resulted in a significant increase in the concentration of these liver biochemical indices when compared to the negative control (Group 2) while the values observed in the positive control (group 3) were observed to be significantly equivalent to the values in the AML and FZR treatment groups.

Figure 7. Total protein concentration in zidovudine induced rats treated with extracts of Annona muricata leaves and Fagara zanthoxyloide roots.
The decrease in the concentration of total protein, albumin and globulin in the negative control (Group 2) when compared to the control suggest that ZDV may induce hepatotoxicity (George, 2009) resulting in increase amounts of p-proteins or decreased antibody production, causing nephrosis and acute hemolytic anaemia (Ekam and Udosen, 2012). The increase in the concentrations of these liver biochemicals in AML and FZR treatment groups may buttress publications that these extracts possess hepatoprotective potentials mediated by antioxidants in Wistar rats (Ekere et al., 2019).

The bilirubin concentration (Figure 10) was observed to increase on the administration of ZDV (Group 2) when compared to the normal control while simultaneous administration with AML and FZR extracts (Groups 4 and 5) resulted in significant reduction (p≤0.05) in the bilirubin concentration at week 2 when compared with the negative control and further reduction as time progressed. The increase in the level of bilirubin may be possibly caused by an increase in haem metabolism (Amadi et al., 2018) as bilirubin has been observed to accumulate from the breakdown of haemoglobin, a key component of red blood cells (Imafidon and Okunrubo, 2012). It may also be a sign of hepatotoxicity displayed as impairment in the excretory function of the liver or a compromise in its overall functioning as the liver is responsible for the removal of bilirubin from the blood and excreting it through the bile (Senou, et al., 2017).

The serum concentrations of urea and creatinine in the negative control group (Table 1) were observed to significantly reduce (p≤0.05) on administration of ZDV in group 2 when compared with the normal control. However, the simultaneous administration with extracts of AML and FZR resulted in a significant increase (p≤0.05) in the serum concentrations of urea and creatinine at week 2, 4 and 6.

Table 1: Kidney indices in zidovudine-induced rats treated with extracts of Annona muricata leaves and Fagara zanthoxyloide roots.

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<tr>
<th>GRP</th>
<th>Urea (mmol/L)</th>
<th>Creatinine (µmol/L)</th>
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<tr>
<td></td>
<td>Week 2</td>
<td>Week 4</td>
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<tr>
<td>Group 1</td>
<td>5.69±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.69±0.20&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>Group 2</td>
<td>4.57±0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.15±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 3</td>
<td>5.24±0.28&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.15±0.04&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 4</td>
<td>5.34±0.02&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>5.47±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 5</td>
<td>5.31±0.03&lt;sup&gt;c,e&lt;/sup&gt;</td>
<td>5.37±0.02&lt;sup&gt;b,c&lt;/sup&gt;</td>
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Values expressed as Mean ± SEM of triplicate determination. Values with same superscript are statistically significant (p<0.05).
The urea and creatinine concentrations have been reported to serve as reliable markers of the functionality of the kidney (Belonwu et al., 2013), as such the decrease in the concentrations would signify that ZDV results in a decrease in the optimal functionality of the kidney possibly resulting in nephrotoxicity as reported by Amadi et al., (2018). The increase in the serum urea and creatinine concentrations on administration with extracts may suggest that the extracts of AML and FZR prevent nephrotoxicity induced by ZDV by a mechanism not explored in this study (Ogunnka-Nnoka et al., 2012).

**Histological examination**

An examination of the liver of group 1 showed the hepatocytes in normal architecture radiating from the portal triad (Plate 1). Administration of 100mg ZDV/Kg B.W resulted in severe dilation, congestion of blood vessel transversing the liver and alterations in the architecture of the hepatocytes which progressed towards necrosis and sinusoidal infiltrations at week 6 (Plates 2-4).

Treatment with *AML* showed hepatocytes with preserved architecture when compared with the negative control. Slight dilation in the hepatic cords and single cell necrosis was also observed on prolonged administration with *AML* (Plates 8-10). Prolonged *FZR* administration resulted in hepatic regeneration at week 4 with a decrease in hepatic infiltration and congestions observed at week 6 (Plates 12 and 16).

Sinusoidal congestion and dilations have been reported by Adisa et al., (2014) as an important marker of early onset hepatotoxicity. The necrosis observed in the negative control group serves as a sign of hepatic degeneration, which in combination with the aforementioned signs may suggest that ZDV may induce hepatic damage at the dosage administered. According to Akinnawo et al., (2005), the architecture for the group administered with *AML* may signify that the extract confers hepatoprotective abilities through the presence of several antioxidants (alkaloids, phenols, tannins), anti-inflammatory agents (norchelerythine, α-humlene, β-pinene, zinc, fiber), myleoregenerative and tissue regenerative agents (amino acids, zinc, calcium, folate, vitamin B₁₂) as seen in Plate 8-9 (Ekere et al., 2019, Adisa et al., 2014). The observations in the group administered with *FZR* may also signify a regression in the observed deleterious effects of ZDV on administration indicating a protective effect conferred on the hepatocytes by the extracts.

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**Plate 1.** Liver of normal control group showing hepatocytes arranged in rolls radiating from the portal triad along with a few dilated hepatic veins.

**Plate 2.** Liver of Negative control group at week 2 showing severe dilation and congestion of blood vessels with altered architecture.

**Plate 3.** Liver of Negative control group at week 4 showing dilation and portal vein congestion.

**Plate 4.** Liver of Negative control group at week 6 showing marked sinusoidal congestion and necrosis.

**Plate 5.** Liver of group 3 at week 2 showing congestion and dilatation in blood vessels.

**Plate 6.** Liver of group 3 at week 4 showing hepatocytic dropout and formation of rosette.

**Plate 7.** Liver of group 3 at week 6 showing hyalinization and progressive lymphocytic infiltration.
The kidney of the normal control group showed normal architecture especially with the renal corpuscles, tubules and connecting ducts (Plate 14). The administration with ZDV resulted in hypoplasia of the cells with swellings observed at the epithelium of the renal tubules. The co-administration with extracts of AML and FZR resulted in the kidneys displaying normal architecture but with slight dilations observed in the glomeruli.

Plates 14. Kidney of group 1 showing kidney cortex with normal structure of renal corpuscles, renal tubules and collecting ducts

Plates 15, 16, 17. Kidney of group 2 at week 2, 4 and 6 showing glomerulus was observed to be hyperplastic with renal tubules epithelium showing signs of swellings

Plates 18, 19, 20. Kidney of group 3 at week 2, 4 and 6 showing collecting tubules lined with the relatively low simple cubic epithelium.
Hyperplasia observed in the negative control group has been linked to a regression in the development of the kidney cells and reported by Adewole et al., (2006) as an early sign of renal adaptations to toxins and possibly nephrotoxicity. The normal architecture observed on co-administration with AML may further buttress the findings of Ekere et al., 2019 which suggested that the extracts contained anti-oxidative agents (alkaloids, tannins, phenols) and anti-inflammatory agents (norchelerythine, α-humlene, β-pinene, zinc, fiber) which confer some degree of protection to developing kidney cells hence imitating nephroprotectivity.

CONCLUSION

The administration of zidovudine resulted in hepatotoxicity and nephrotoxicity which were observed to be reduced to some degree when extracts of AML and FZR were administered as seen with the results of histological investigations and liver and kidney indices investigated. The impact of the extracts on these liver and kidney indices and body and organ weight may suggest that these plants may be hepatoprotective and nephroprotective to some degree and may also be used in anorexigenic conditions to boost body weight.

REFERENCES


