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HISTOLOGICAL AND BIOCHEMICAL MARKERS OF THE LIVER OF MALE WISTAR RATS ON ORAL ADMINISTRATION OF NEVIRAPINE SUSPENSION

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ABSTRACT

Background: Mechanism of action of nevirapine in the prophylaxis treatment and treatment of HIV-1 may involve elevations in levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and other biomarkers of liver function. This study presents the hepatotoxic effect of nevirapine suspension using animal model.

Methods: A total number of 15 male Wistar rats were fed normal chow and antiretroviral drug (Nevirapine) for a period of six weeks. The liver organ of the rats were obtained and subjected to histological procedures and biochemical analysis using enzyme assay obtained from Randox Laboratories Limited, Antrim United Kingdom (BT294QY).

Results: The wistar rats showed no significant mean body weight difference when compared with the control group. However there was significant difference in the mean values of AST (77.77 ± 3.03) and ALT (89.37 ± 3.19) of the treated rats. Nevirapine treated rats showed significant difference in AST, ALT, and ALP in the single (77.77 ± 3.03 , 31.80 ± 1.73 , 43.81 ± 1.54) and double (89.37 ± 3.19 , 33.38 ± 2.01 , 34.64 ± 1.02) doses when compared with the controls (75.14 ± 2.00 , 29.16 ± 0.17 , 45.44 ± 1.85) respectively. Mild vascular congestion, infiltration of sinusoids by inflammatory cells, and haemorrhage were induced by nevirapine as compared with the control group showing normal vessels without congestion, normal sinusoids appearing normal without infiltration.

Conclusion: The liver histology of the rats fed with Nevirapine suspension showed diffused hepatocellular necrosis. Routine check of the drug effect is important as it provides effective life management of HIV infected individuals.

Keywords: Nevirapine, Wistar rat, Hepatotoxicity, Liver, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP).

HISTOLOGIQUES ET MARQUEURS BIOCHIMIQUES DU FOIE DE RATS MALES WISTAR PAR ADMINISTRATION ORALE DE LA NEVIRAPINE SUSPENSION

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RÉSUMÉ

Contexte: Mécanisme d'action de la névirapine dans le traitement de prophylaxie et le traitement du VIH-1 peut impliquer élévations des taux d'alanine aminotransférase, aspartate aminotransférase, la phosphatase alcaline et d'autres biomarqueurs de la fonction hépatique. Cette étude présente l'effet hépatotoxiques de la suspension de la névirapine en utilisant un modèle animal.

Méthodes: Un nombre total de 15 rats mâles Wistar ont été nourris chow normal et médicament antirétroviral (névirapine) pour une période de six semaines. L'organe du foie des rats ont été obtenus et soumis à des procédures histologiques et analyse biochimique utilisant un dosage de l'enzyme obtenue à partir de Randox Laboratories Limited, Antrim Royaume-Uni (BT294QY).

Résultats: Les rats Wistar ont montré aucune différence significative de poids corporel moyen en comparaison avec le groupe témoin. Cependant il y avait de différence significative dans les valeurs moyennes d'AST ($77,77 \pm 3,03$) et ALT ($89,37 \pm 3,19$) des rats traités. Névirapine chez les rats traités ont montré de différence significative dans AST, ALT et ALP dans la seule ($77,77 \pm 3,03$, $31,80 \pm 1,73$, $43,81 \pm 1,54$) et double ($89,37 \pm 3,19$, $33,38 \pm 2,01$, $34,64 \pm 1,02$), des doses en comparaison avec les contrôles ($75,14 \pm 2,00$, $29,16 \pm 0,17$, $45,44 \pm 1,85$), respectivement. Légère congestion vasculaire, infiltration des sinusoides

par des cellules inflammatoires, et des hémorragies ont été induites par la névirapine par rapport au groupe de contrôle montrant les vaisseaux normaux sans congestion, sinusoides normales apparaissant normale sans infiltration.

Conclusion: L'histologie hépatique des rats nourris avec névirapine suspension a montré une nécrose hépatocellulaire diffuse. Contrôle de routine de l'effet du médicament est importante car elle permet une gestion efficace de la vie des personnes infectées par le VIH.

Mots-clés: Névirapine, Wister rat, Hépatotoxicité, Foie, de l'alanine aminotransférase (ALT), d'aspartate aminotransférase (AST), la phosphatase alcaline (ALP).

INTRODUCTION

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used for prophylaxis and treatment of Human Immunodeficiency Virus (HIV) infections [1,2]. It acts by reversibly inhibiting the activity of HIV-1 reverse transcriptase, an enzyme which directs the polymerization of DNA from viral RNA, a necessary component for HIV-1 replication [3]. The inhibition of reverse transcriptase-directed polymerization of DNA from viral RNA has been an important therapeutic target for the treatment of HIV-1 infection, which was initially reported with the nucleoside analogue AZT [4]. Unlike the nucleoside analogues, NVP binds directly to the reverse transcriptase at amino acid residues 181 and 188. This site is close to but not directly at the polymerase catalytic site on the large subunit of the heterodimeric reverse transcriptase. The binding of NVP to reverse transcriptase occurs primarily through hydrophobic interactions to a pocket formed by seven strands, as a result the rate of the chemical reaction catalysed by the reverse transcriptase is significantly slowed [5]. NVP does not exhibit activity against other viral polymerase, including HIV-2 and simian immunodeficiency virus reverse transcriptase. NVP has been studied in several combination regimens for the treatment of HIV [6].

As nevirapine dose administered to mother and infant has been widely used to prevent mother-to-child transmission of HIV-1 in resource-limited settings [7], it has also been associated with severe skin and hepatic hypersensitivity reactions that have hampered its use particularly for HIV prophylaxis [8]. Hepatotoxic effect of NVP is common in patients with higher CD4 counts and also in the first three weeks of NVP treatments in HIV infected subjects [9,10]. The immune pathways that consequently cause the liver damage have been likened to the pathogenesis of liver injury in diseases such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infections where activated cell-mediated immunity is incriminated for the liver damaged [11,12]. The fact that NVP-induced hepatotoxicity is common in patients with higher CD4 counts imply that increased stimulation of the cell-mediated immune system response in some HIV-positive patients may predispose them to NVP-induced hepatotoxicity. According to Stern *et al.*, [13] and Dieterich *et al.*, [14], the mechanism of NVP-induced hepatotoxicity remains unknown,

however, it was postulated to be immune mediated. Such immune mediation has already been proven in animal models for NVP-induced skin reactions [15,16]. Hepatotoxicity, a case of liver dysfunction or liver damage, is sometimes associated with an overload of drugs or xenobiotics, producing a wide variety of clinical and histopathological indicators of hepatic injury, however, the measurement of some level of substances that may be present in the blood helps in initial detection [17,18,19].

Several enzymes that trigger important chemical reactions in the body are produced and found within the cells of the liver, however, damage or injury to the liver cause elevations to the liver enzyme levels. These enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function [20]. Other measurable liver function is reflected in albumin and total protein concentration and the prothrombin time, which are the markers of liver biosynthetic capacity [3,5].

As HAART can substantially extend an HIV patient's life, one of the major problems are its adverse systemic and oral effects. To examine the mechanism of effect of nevirapine, an examination of the histological and biochemical markers of liver using wistar rats administered nevirapine were compared to those who were not given for a period of six weeks.

MATERIALS AND METHODS

Animals

Fifteen male adult wistar rats were obtained from a breeding stock maintained in the animal house of the Agricultural Science Department, Ladoké Akintola University of Technology (LAUTECH), Ogbomoso, and housed in well ventilated plastic cages in animal house in Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The rats were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e. L:D;12h:12h photoperiod) at room temperature, allowed unrestricted access to water and rat chow and acclimatized for 7 days before the

commencement of the experiment. The body weights of the rats ranged between 180 and 300g.

Drugs and Source

The antiretroviral drug (Nevirapine) used was produced for Evans Medical Plc Nigeria by CIPLA Limited, Verna Indl. Estate Goa 403 722 India with Batch No.G10930 and National Agency for Food Drug Administration and Control (NAFDAC) reg. No. 04-9498.

Experimental procedure

Fifteen male wistar rats were randomly distributed into three groups with five rats per group. Corresponding therapeutic doses for rat models were calculated and aqueous solutions formed were administered daily as follows. Rats in groups 1 (Control) received 0.9% food and normal saline. Group 2 (single dose) received 0.01% of Nevirapine, food and normal saline. Group 3 (double dose) received double dose of Nevirapine, food and normal saline.

Animal sacrifice and sample extraction

Animals were weighed and sacrificed after six weeks treatment. Blood samples were obtained by cervical dislocation and collected into EDTA bottles. Serum was used for the hepatic enzymes activities (ALT, AST and ALP).

Histological Procedures and Analysis of the liver

The liver organs were cut on slabs (0.5cm thick) and fixed in 10% formol saline for a day after which

they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before transferred into two changes of molten paraffin wax for 20min each in an oven at 57°C. Serial sections of 5µm thick were obtained from a solid block of tissue and stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven-dried. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK) to demonstrate the cytoarchitecture of the liver.

Biochemical Analysis

The analysis of the result for ALT, AST and ALP were done using SPSS program for windows (17.0 version). The reagents used for the enzyme assay were obtained from the Randox Laboratories Limited, Antrim United Kingdom (BT294QY).

RESULT

The mean body weight gain of the rats treated with antiretroviral drug as shown in Table 1 has no significant differences when compared with the control group that received distilled water and food for 6 weeks.

Table 2 shows significant differences in ALP, AST and ALT. AST and ALT mean values increased as compared to the control, however, ALP mean value decreased when compared with the control.

TABLE 1: MEAN BODY WEIGHT OF RAT

Group/Day	1-7	8-14	15-21	22-28	29-35	36-42
1	158.09 ± 0.39	166.07 ± 0.43	154.04 ± 0.34	124 ± 0.17	144.04 ± 0.25	157.06 ± 0.34
2	159.06 ± 0.41	142 ± 0.21	156 ± 0.38	164.05 ± 0.42	138 ± 0.28	140.02 ± 0.20
3	160 ± 0.38	145 ± 0.30	155.04 ± 0.35	200.6 ± 0.46	240.03 ± 0.51	234 ± 0.48

Key: Group 1- Control Group 2 - Single dose Group 3-Double dose

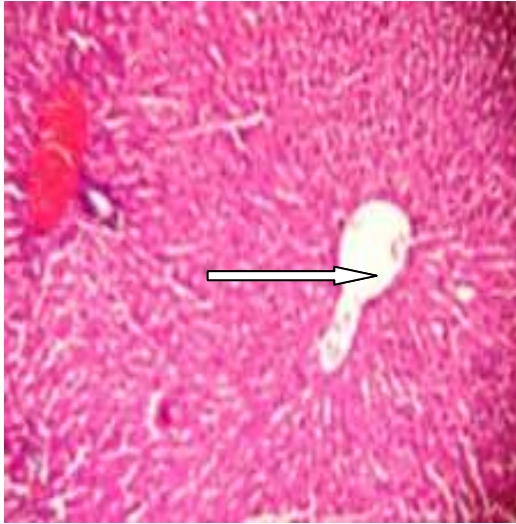
TABLE 2: EFFECTS OF NEVIRAPINE ON LIVER FUNCTIONS

Biochemical Markers	Group1	Group2	Group3
AST (U/L)	75.14 ± 2.00	77.77 ± 3.03	89.37 ± 3.19
ALT (U/L)	29.16 ± 0.17	31.80 ± 1.73	33.38 ± 2.01
ALP (U/L)	45.44 ± 1.85	43.81 ± 1.54	34.64 ± 1.02

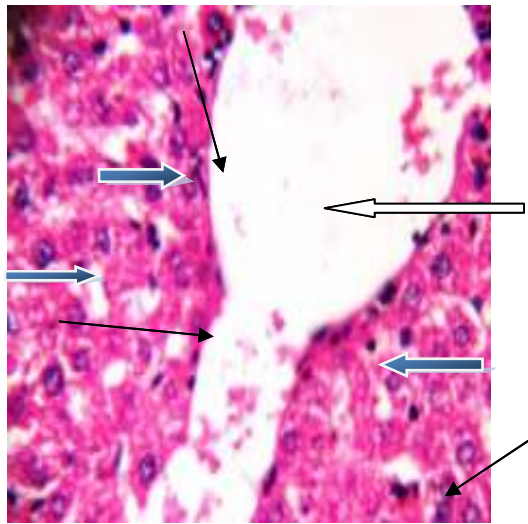
Key: Group 1-control Group 2-single dose Group 3-Double dose.

Plates 1, 2 and 3 show the gross morphology of liver for rats fed with NVP as compared with the control. Those fed with NVP suspension showed diffused

hepatocellular necrosis in about 90% of the rats. No such changes were observed in the control.



X100

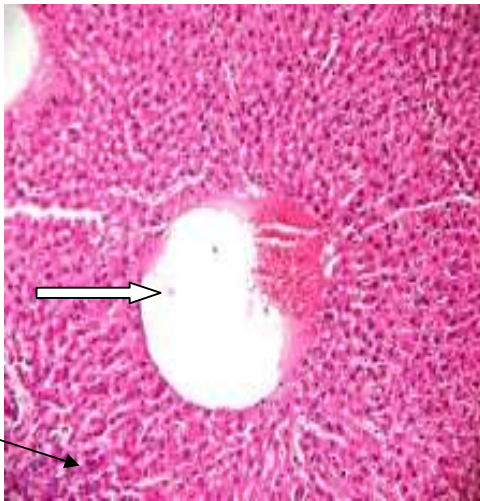


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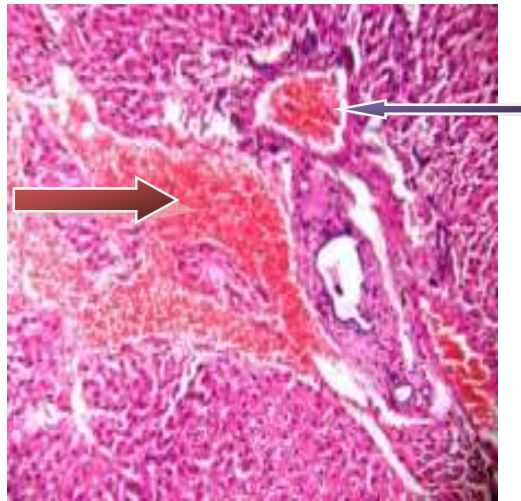
PLATE 1: MICROGRAPH OF LIVER SECTION OF RATS IN THE CONTROL GROUP

Normal vessels without congestion (white arrow), the sinusoids (slender arrow) appear normal without infiltration, the hepatocytes show normal

morphology (blue arrow). No pathological lesion seen.



X100



X100

PLATE 2: MICROGRAPH OF LIVER SECTION OF RATS FED WITH SINGLE DOSE OF NEVIRAPINE SUSPENSION

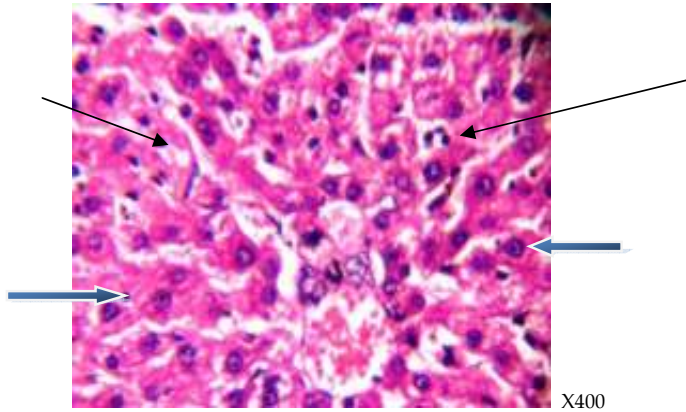


PLATE 2(CTD.): MICROGRAPH OF LIVER SECTION OF RATS FED WITH SINGLE DOSE OF NEVIRAPINE SUSPENSION

The central vessel appear normal (white arrow), there is mild congestion of the portal vein (black arrow) and focal area of mild haemorrhage (red

arrow), the sinusoids shows mild infiltration of inflammatory cells (slender arrow) the hepatocytes show normal morphology (blue arrow).

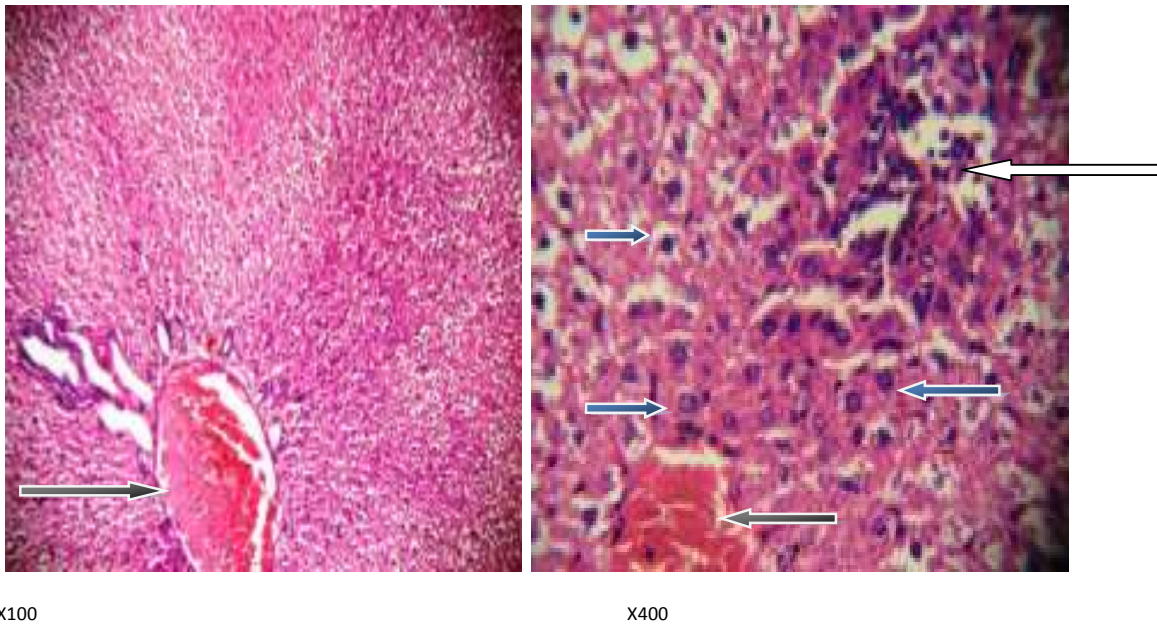


PLATE 3: MICROGRAPH OF LIVER SECTION OF RATS FED WITH DOUBLE DOSE OF NEVIRAPINE SUSPENSION

There is mild vascular congestion (black arrow), the sinusoids show mild infiltration by inflammatory cells, there is focal granuloma within the liver parenchyma (white arrow), the hepatocytes show normal morphology (blue arrow).

antiretroviral drugs in the treatment of human immunodeficiency virus infections. Its mechanism of action is not limited to treatment of HIV-1 but also in the reduction of mother-to-child transmission of HIV-1 which may involve reduction of maternal viral load as well as prophylaxis of infants [2]. The drug can be used as a single-dose or as a combination therapy with other antiretroviral drugs including lamivudine, stavudine and Zidovudine [21,22,23,24]. Nevirapine administration has been associated with severe skin and hepatic hypersensitivity reactions that have

DISCUSSION

Effects of administration of NVP given orally on morphology of the liver of albino wistar rats were studied. Nevirapine is one of the most widely used

hampered its use particularly for HIV prophylaxis [25,26,8] as well as oral adverse effects, including whitish plaque in the lips and bilateral buccal mucosa, burning, taste disturbance, and xerostomia [1].

From this study, no significant difference was recorded in the mean body weight of the rats fed with NVP as compared with those in the control group. This supports the findings from a similar study by Ayeni *et al.*, [24].

As revealed from the result in this study, Nevirapine is associated with significant activities of ALT and AST. Elevated activities of these enzymes indicate hepatic damage which results from several mechanisms including generation of toxic species and peroxidation of membranes [21]. The results showed that AST mean values were significantly increased in rats treated when compared with the control values. This elevation in Nevirapine treated rats agrees with earlier reports of Sule *et al.*, [23], Johnson and Baraboutis [27] and Martinez *et al.*, [9]. However, Nevirapine treated rats showed significant reduction in the values of ALP when compared with the control. According to Dufour *et al.*, [28,29], ALT activity is the most frequently relied biomarker of hepatotoxicity in that it plays a vital role in amino acid metabolism and gluconeogenesis. The estimation of this enzyme is more specific for liver abnormalities since it is primarily located in the liver [30]. Aspartate aminotransferases (AST) is another liver enzyme found in the liver and other organs including heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level [30]. It helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury [31]. It can also signify abnormalities in heart, muscle, brain or kidney [28,29]. According to Nathwani *et al.*, [30], the ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage. Alkaline phosphatase (ALP) may be elevated if bile excretion is inhibited by liver damage. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepatobiliary effects and cholestasis [32]. In humans, increased ALP levels have been associated with drug-induced cholestasis [33]. As revealed from the result in this study, nevirapine is associated with significant elevated activities of ALT and AST. The result shows that AST mean values were significantly increased in rats treated with the antiretroviral drug when compared with the control values, which agrees with earlier reports of Umar *et al.* [21], Sule *et al.*, [23], Johnson and Baraboutis [27] and Martinez *et al.* [9]. They concluded that nevirapine could be associated with hepatotoxicity. Nevirapine hepatotoxicity could be associated with some risk factors including gender,

CD4 cell count, co-infection with hepatitis B or C and pregnancy [34,35,36,37,2,38]. Several drugs known to induce hepatotoxicity, like nevirapine, in association with an activated immune system include diclofenac [39], paracetamol [40], bacterial lipopolysaccharide (LPS) plus ranitidine [41] and trovafloxacin [42]. The elevation of enzymes activity, especially in ALT in Nevirapine-treated rats is indicative of liver injury. This agrees with reports that severe hepatic reactions of HAART was attributed to Nevirapine component of HAART [27,9]. Sulkwosk *et al.*, [43] also reported hepatotoxicity as a major side effect of all antiretroviral classes with Nevirapine having the highest risk. Liver converts drugs into reactive forms and hence results in toxicity [21].

Histological examination of NVP administration on the morphology of some organs of the body have been reported and series of reports as regards the toxicity of NVP a non-nucleoside reverse transcriptase inhibitor on the small intestine, kidney, spleen, mitochondria, bile, muscle and bone had been recorded [44,45,46,47,24]. The gross morphology of the liver from rats fed NVP observed in this study agrees with that reported by Umoren and Osim [3]. From result of this study as shown in Plate 1 revealed a normal healthy state of the liver with the portal tract intact. There are normal vessels without congestion, periportal hepatocytes arranged in plates and sinusoids appeared normal without infiltration, within the portal tract were the portal vein, hepatic artery and bile duct, no pathological lesion is seen. From plate 2, the liver of the rats showed disorganized cyto-architecture with sinusoidal and central vein endothelial desquamation. It revealed mild congestion of the portal vein and focal area of mild haemorrhage, the sinusoids showed mild infiltration of inflammatory cells, mild perivascular infiltration, the sinusoids are mildly infiltrated by inflammatory cells even though the hepatocytes showed normal morphology. Plate 3 revealed poor architecture of the liver. There was mild vascular congestion with the sinusoids showing mild infiltration by inflammatory cells. A focal area of granuloma within the liver parenchyma was seen. There was mild congestion of the portal vein which shows that much damages was done in the liver of the rat administered with double dose of Nevirapine. The present result agrees with studies of Degott [48] who reported that liver damage is associated with alteration in bile secretion and Akerlund *et al.*, [49] who showed that there is always an increase in cholesterol synthesis when there is a disturbance in bile release and utilization due to liver damage.

Umoren and Osim [3] earlier reported the effects of NVP administration given through oral gavage on biliary secretion and its biochemical composition in

albino wistar rats. The result obtained showed significantly decreased biliary secretions resulting in insignificant increase in conjugated bilirubin but significant elevations in total cholesterol, total bilirubin, and unconjugated bilirubin. Also, significant decreases in biliary electrolytes concentrations were observed.

Furthermore, Poirier *et al.*, [50] and Ayeni *et al.*, [24] reported that NVP can cause acute kidney injury as a result of severe mitochondrial dysfunction and lactic acidosis induced as well as acute renal failure after the initiation of NVP during the study of the effects of NVP on foetal parameters, kidney and spleen of dams.

Conclusion

The above results suggest that NVP administration may cause liver hepatotoxicity in albino wistar rats.

The effect of antiretroviral drugs on biochemical indices of liver function is of paramount importance and should not be overlooked. This is to ensure that the liver function is not impaired in the process of managing a particular health problem in case of HIV patient, hoping to minimize the duplication of this virus in the human system, while a lot of harm is being done to the liver. The observation that it takes some weeks to develop liver injury means that NVP itself plays a role in the initiation of the lesion. Therefore, it can be concluded that NVP activates the cell-mediated immune response leading to liver injury that is propagated by the drug itself or the immune system.

Following the results in this study and various reports on related studies, it could be concluded that the lives of HIV patients on regular use of HAART containing Nevirapine are prone to risk.

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