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Evaluation of HIV1 GP 120-CD4 Binding Inhibition Potentials of the Stem Bark Extracts of *Diospyros mespiliformis*

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Abstract

The study was conducted to evaluate the HIV1 gp120-CD4 binding inhibitory potential of crude aqueous, methanol and petroleum ether extracts of Diospyros mespiliformis. The extracts were obtained by Soxhalet extraction. Phytochemical screening, gp120-CD4 binding inhibitory potential, and sub-acute toxicity tests were carried out using standard techniques. Total of six phyto-constituents were identified in the extracts; flavonoids, alkaloids and balsams in aqueous extract, while flavonoids, alkaloids, tannins, balsams, steroids and cardiac glycosides in methanol extract, and flavonoids, alkaloids in the petroleum ether extract. Mean percentage inhibitions of the extracts of Diospyros mespiliformis against HIV-1 gp120-CD4 binding were recorded at various concentrations of the extracts, with 10% inhibition was recorded at 125µg/ml in aqueous extract, 13% and 18% inhibitions were recorded at 250 and 125µg/ml in methanol extract respectively, while 3% inhibition was found in 125µg/ml of petroleum ether extract respectively. Physical signs of toxicity; weight changes, hair loss, diarrhea, and weakness of the body of the laboratory animals treated with 250 and 125mg/kg were observed. There was no significant difference (P>0.05) in serum Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP). There were no signs of Inflammation observed in the tissues of animals treated with all the extracts. It can be concluded that the methanol extracts possessed higher anti gp120-CD4 binding activity and extracts were non-toxic to the animals.

INTRODUCTION

Seven million people have been infected with HIV-1 since the beginning of the epidemic in 1981, and about 32 million are now deceased (WHO, 2018). Due to the poor health systems and living conditions, developing countries are the hardest hit by this epidemic. The WHO African region remains most severely affected, with nearly 1 in 25 adults living HIV and accounting for more than two-thirds of the people living with the virus worldwide (WHO, 2018). Sub-Saharan Africa, is where the majority of new HIV-1 infections occur, these countries are home to 25.8 million people infected with HIV-1, accounting for almost 70% of the global HIV-1 infected population (Ayesha *et al.*, 2016) and there are 1.9 million infected in Nigeria (UNAIDS, 2019).

Despite all the available pharmaceuticals for the treatment of HIV, there is still no cure for the deadly disease and HIV viruses continue to mutate and become resistant to existing drugs. The medical communities throughout the world continue to search for drugs that can prevent HIV infections, treat HIV carriers to prevent them from progressing to full-blown AIDS and treat the AIDS patient. Currently no much has been done on the search for HIV drugs especially from savannah plants that can inhibit the binding of the virus to the susceptible cells (CD4 Lymphocytes), which is an important step in the replication cycle of HIV. The available drugs for the treatment of HIV are expensive and toxic to some extent; hence the need to test some of the Savannah plants for their anti HIV potential.

The *Diospyros mispiliformis* also known as Monkey Guava or Jackalberry, or West African Ebony is an evergreen tree that reaches up to 20m in height, or up to 45m in forests. West African Ebony has a wide range of medicinal uses. Different plant parts can be made into variations and used in the treatment of a range of conditions like fever, pneumonia, dysentery, syphilis, leprosy, yaws, menorrhoea, diarrhoea, headaches, arthritis, gingivitis, toothache, cuts and wounds, otitis, stomach pains, sores, ulcers, etc.

The principle constituent appears to be plumbagin, which has been shown to have antibiotic, antihaemorrhagic and fungistatic properties. It is found in the root-bark to a concentration of 0.9% and but a trace in the leaves (Sharma, 2017). Tannin, saponin and a substance probably identical to scopolamine are also present (Sharma, 2017).

The antiviral activity of the plant has not been fully exploited despite some claims by traditional medicine practitioners that the plant possesses some antiviral activity. Therefore, there is growing need to evaluate *Diospyros mespiliformis* for its gp120-CD_4 inhibition potential against HIV1.

MATERIALS AND METHODS

Collection of Samples

Fresh stem bark was collected from *Diospyros mespiliformis* tree in April, 2018 from Karaye LGA, Kano. The plant was re-identified at the Department of Plant Biology, Bayero University, Kano, where a herbarium number "BUKHAN 121" was assigned to it. The stem bark was air-dried for ten days and then ground in to powder and sieved thoroughly using 0.1mm mesh. The powder was kept in a non-absorptive container before use.

Extraction of Phytoconstituents

The Phytoconstituents were extracted using distilled water, pet-ether and methanol (96%) as solvents by Soxhlet extraction protocols as described by Tariq *et al*. (2011). Fifteen grammes of the powdered plant material was wrapped in Whatman filter paper and inserted in to thimble of a Soxhlet extractor which was connected using the manufacturers' guidelines, and then 300ml of the solvent was heated to boiling. The vapour passed through coils of cool water in the extractor, which enable it to condense and then drop on the plant material. This allows extraction of the soluble components of the plant material. The process is repeated several times until when the soluble components are maximally extracted.

Phytochemicals Analyses

Some physicochemical characteristics of the extracts were investigated (colour, texture, odour and pH) the extracts were also analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars as described by Adetuyi and Popoola, 2001; Trease and Evans,1989; Sofowora, 1982.

Screening for Anti gp120-CD4 Binding Potential of the Stem Bark Extract using (Human Immunodeficiency Virus type 1 (HIV-1) gp120/Glycoprotein 120 ELISA Kit Beijing China)

Three extracts; aqueous, methanol and petroleum ether extracts were used to prepare four (4) different concentrations (1000, 500, 250, and 125µg/ml) respectively in 4 sets of test tubes using dilution buffer and labeled accordingly. The micro plate wells Sigma-Aldrich, St. Louis, MO, USA were labeled 1-8 vertically representing the four different concentrations in duplicate (1-2 for 1000µg/ml, 3-4 for 500µg/ml, 5-6 for 250µg/ml, and 7-8 for 125µg/ml) of the extracts, while the horizontal axis was also labeled A-D for the control and different types of extracts (A= Control, B= Aqueous, C=Methanol, and D= petroleum ether stem bark extracts of *Diospyros mespiliformis*). Each well was washed three times with wash buffer (300µL/well) using autowasher. The wells were then aspirated to remove the remaining wash buffer (PBS containing 0.1% Tripton- X). The plate was inverted and blotted dry with a clean paper towel. One hundred microlitres (l00µL) of different concentrations from different extracts were added to the respective wells within 15minutes without interruption. The plate was covered and incubated at room temperature for 2 hours. The washing and aspiration was then repeated as earlier done. One hundred microlitres (100µL) of detection antibody in working concentration was added to each well. The plate was covered and incubated at room temperature for 1 hour. Then, two hundred microlitres (200µL) of substrate solution (mixture of colour reagent A and B) were added to each well and incubated for 20 minutes at room temperature and protected from light. After the incubation, 50µL of stop solution was added to each well and the plate was tapped to ensure thorough mixing. The optical density of each well was read after 20 minutes using a micro plate ELISA reader at 450nm. Percentage gp 120-CD4 inhibition was then calculated below as described by Rege *et al*. (2010).

$$\text{Percent inhibition} = \frac{\text{Absorbance of the sample} - \text{Absorbance of the control}}{\text{Absorbance of the control}} \times 100$$

Sub-Acute Toxicity Study Using Albino Rats

The rats were sourced from animal room of Department of Biological Science Bayero University, Kano and allowed to acclimatize to the test environment for 7 days during which they were fed with clean water and food. The animals were grouped in to 4 of 4 animals each with the exception of the last group with only 2 animals as negative control to make the total of fourteen (14) rats (weighing 100-106g weight range) for each of the three extracts; aqueous, methanol and petroleum ether extracts of the plant sample. The rats in group I were orally administered 250mg/kg (lower) concentrations of aqueous extract and the other two also administered with 500mg/kg (higher) concentrations of the aqueous extract. The same procedure was repeated for the group II (methanol extract) and group III (petroleum ether) extracts respectively.

The remaining two rats were used as negative control by being administered distilled water. During the test, 1.0 ml of each extract concentration was administered orally to each rat and distilled water to each control rat daily for a period of 4 weeks. The rats were then observed for possible physical change(s) on weekly basis for 28 days.

At the end of 28 days, the animals were anaesthesized by dropping each of the animals in a transparent plastic jar saturated with chloroform vapour. The anaesthesized animals were then removed from the jar and blood samples collected through cardiac puncture. Samples were collected in lithium heparin blood bottles, the blood samples were mixed gently, then centrifuged and serum collected for biochemical analyses. While the fresh organs; Liver, Lungs and Kidneys were dissected out of each rat and fixed in 10% formalin saline for histological investigation.

Biochemical Analyses of the Blood Samples of Swiss Albino Rats.

Biochemical parameters sodium ions, potassium ions alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, createnine, serum albumin, total proteins, total bilirubins, urea, and bicarbonate ions were determined as described by Cheesbrough (2000).

Histological Examination of Some Organs of Swis Albino Rats

Liver, kidney, and lung tissues of the test albino rats were fixed with 10% formalin saline, dehydrated with ascending grade of alcohol, cleaned with toluene, and then infiltrated with molten paraffin wax. The microtome section was stained with haematoxylin and eosin staining technique examined with Leica DM 75 microscope and then photographed with Leica ICC 5 HD camera (Auwioro, 2010).

Statistical Analysis

The results were analyzed as mean percentages.

RESULTS

Physico-chemical Properties and Phytochemical Constituents of Stem Bark Extract of *Diospyros mespiliformis*

Results showed that the crude aqueous, methanol and petroleum ether extracts appeared dark brown, brown and yellow in colour respectively. All the extracts were gummy in texture and odourless, the pH ranged from 6.2-7.1 (Table 1).

Six phytochemical constituents including flavonoids, alkaloids, and balsams were present in aqueous extract, while flavonoids, alkaloids, tannins, balsams, steroids and cardiac glycosides were present in methanol extract, only flavonoids and alkaloids were present in petroleum ether extracts (Table 2).

gp120-CD4 Binding Inhibition Potentials of Stem Bark Extracts

The Results showed mean percentage inhibition of the extracts of *Diospyros mespiliformis* against HIV-1 gp120-CD4 binding, where 10% inhibition was recorded in 125µg/ml of aqueous extract, whereas, 13% and 18% inhibitions were recorded at 250 and 125µg/ml in methanol extract respectively, while 1% inhibition was found in 125µg/ml of petroleum ether extract (Table 3).

Sub Acute Toxicity Study of Stem Bark Extract of *Diospyros mespiliformis*

There was no hair loss, weight loss, diarrhea or death recorded in the rats after 28 days period of the study (Table 4).

Liver Function Tests

Total protein and A:G ratio were found to be within the normal range in all the extract, but there is a significant increase in albumin and globulin concentration at methanol high concentration extract (500mg/kg) (Table 5).

While Table 6, is the liver function test determining Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) of the test rats' sera at 500mg/kg and 250mg/kg of aqueous, methanol, and petroleum ether extract including control respectively. There was a significant increase in AST and ALP in all the extracts both the low and high concentrations. But, ALT concentration values remained within the acceptable range.

Table 7, is the liver function test in which total and direct bilirubin are determined respectively. There is no any significant increase in both the total and direct bilirubin concentration of the rats' sera.

Kidney Function Test

Table 8, presents the results of kidney function test where serum electrolytes ($Na^{+,}$ K^{+}, HCO_3^{-}) are determined and no significant change seen in all the parameters. All parameters found to be within the normal range (Table 8).

Kidney function test results showed that urea level was elevated; however, creatinine concentrations were within the normal range in all the extracts (Table 9).

Table 1: Physical Characteristics of the Stem Bark Extract of *Diospyros mespiliformis*

Phytoconstituent	Aqueous extract	Methanol extract	Petroleum ether
Flavonoids	+	+	+
Alkaloids	+	+	+
Saponins	-	-	-
Tannins	-	+	-
Balsams	+	+	-
Glycosides	-	-	-
Steroids	-	+	-
Cardiac glycoside	-	+	-
Phenols	-	-	-
Anthocyanins	-	-	-

Histological Observation

Plates 4, present the histopathological observations of the liver, kidney, and lung tissue autopsy of the test rats of the different extracts at various concentrations. There are signs of inflammations seen in the plates of tissues treated with methanol extract, but all the remaining tissues treated with other extracts shown no any sign of inflammation.

Table 2: Phytochemical Constituents of Extracts of *Diospyros mespiliformis*.

Extracts	Colour	Texture	Odour	pH
Aqueous extract	Dark brown	Gummy	Odourless	7.13
Methaol extract	Brown	Gummy	Odourless	6.31
Pet. ether extract	Yellow	Gummy	Odourless	6.22

KEY: + Positive, - Negative

Table 3: Mean Absorbance and Percentage Inhibition of Extracts of *Diospyros mespiliformis* Against HIV1 gp120-CD4 Binding at Various Concentrations

	Concentration (µg/ml)			
Extracts	1000	500	250	125
Control	0.146±0.005(0%)	0.146±0(0%)	0.146±0(0%)	0.146±0.005(0%)
Aqueous	0.146±0.005(0%)	0.146±0(0%)	0.146±0.001(0%)	0.141±0.005 (10%)
Methanol	0.146±0.005(0%)	0.146±0.005(0%)	0.128±0.005(13%)	0.120±0.005 (18%)
Petroleum ether	0.146±0.005(0%)	0.146±0.005(0%)	0.146±0.005 (0%)	0.145±0.005 (3%)

Table 4: Sub-Acute Toxicity Effects of Extract of *Diospyros mespiliformis*on Swiss Albino Rat

Extract	Conc. (mg/kg)	Mean Initial weight	Mean final weight	Mean weight difference	% mean weight difference	Death	Hair loss	Diarrhea
Control	250	100.5	104	3.5	3.5	N	N	N
	500	101	105	4.0	4.0	N	N	N
Aqueous	250	100.5	104.5	4.0	4.0	N	N	N
	500	101	104	3.0	3.0	N	N	N
Methanol	250	100.5	103	2.5	2.5	N	N	N
	500	101	102.5	1.5	1.5	N	N	N
Pet – ether	250	102	105.5	3.5	3.4	N	N	N
	500	102.5	106	3.5	3.4	N	N	N

NB: The oral administration time interval was 24hours for 28days period
KEY: Y=Yes, N= No

Table 5: Liver Function Tests for Rats Dosed with Different Extracts at Various Concentrations of *Diospyros mespiliformis* Stem Bark

Extract	Dose (mg/kg)	Concentration of Parameters (g/dl)			
		Total Protein	Albumin	Globulin	A:G Ratio
Control	distilled water	5.884	3.824	2.00	1.912
Aqeous HC	500	6.372	4.706	2.334	2.016
Aqeous LC	250	5.372	3.647	1.875	1.945
Methanol HC	500	5.093	6.118	5.025	1.218
Methanol LC	250	5.697	3.176	3.479	0.912
Pet. Ether HC	500	5.581	4.647	2.866	1.621
Pet. Ether LC	250	5.442	4.118	2.676	1.538

NB: Normal range adopted from Cheesbrough, (2003)
1. Protein 5.2-9.1g/dl
2. Albumin 3.5-5.0g/dl
3. Globulin 2.0-3.5g/dl
4. A:G Ratio 0.8-2.0g/dl

KEY:
HC= High Concentration
LC= Low concentration

Table 6: Liver Function Tests for Rats Dosed with Different Extracts at Various Concentrations of *Diospyros mespiliformis* Stem Bark

Extract	Dose mg/kg	Parameters (Concentration in U/l)		
		AST	ALT	ALP
Control	0	10	6	9
Aqeous HC	500	16	8	10
Aqeous LC	250	16	8	10
Methanol HC	500	49	12	37
Methanol LC	250	41	4	26
Pet. Ether HC	500	47	8	30
Pet. Ether LC	250	36	4	23

NB:AST Normal values is up to 12 U/L, ALT Normal values is up to 12 U/L and ALP Normal values is up to 12 U/L
Normal range source: Cheesbrough (2003)
KEY:
HC= High Concentration
LC= Low concentration

Table 7: Kidney Function Tests for Rats Dosed with Different Extracts at Various Concentrations of *Diospyros mespiliformis* Stem Bark

Extract	Dose mg/kg	Concentration of Parameters (mEq/L)		
		Na^+	K^+	HCO_3^-
Control	0	140.60	4.80	18.75
Aqeous HC	500	137.00	4.67	20.83
Aqeous LC	250	137.70	3.53	20.90
Methanol HC	500	138.45	4.67	21.05
Methanol LC	250	137.10	3.83	20.79
Pet. Ether HC	500	141.60	4.69	21.66
Pet. Ether LC	250	142.65	3.55	21.86

NB:Normal range source: Cheesbrough (2003)
K= 3.4-5.3 mEq/l, Na= 135-155 mEq/l,
HCO_3^- = 20-32 mEq/l
KEY: HC= High Concentration, LC= Low concentration

Table 8: Effect of Various Concentrations of Extracts of *Diospyros mespiliformis* on Kidney Urea and Creatinine

Extract	Dose mg/kg	Concentration of Parameters	
		Urea(mmol/l)	Creatinine(mg/dl)
Control	0	1.965	10.00
Aqeous HC	500	9.390	9.529
Aqeous LC	250	9.064	7.706
Methanol HC	500	13.761	9.647
Methanol LC	250	13.164	8.941
Pet. Ether HC	500	8.964	9.235
Pet. Ether LC	250	8.666	8.765

NB:Normal range source: Cheesbrough (2003)
Urea= 1.7-9.1 mmol/l, Creatinineis up to= 20 mg/dl
KEY: HC= High Concentration, LC= Low concentration

Table 9: Effect of Various Concentrations of Extracts of *Diospyros mespiliformis* on Kidney Total Bilirubin and Direct Bilirubin

Extract	Dose mg/kg	Concentration of parameters (mg/dl)	
		Total bilirubin	Direct bilirubin
Control	0	0.116	1.040
Aqeous HC	500	0.256	1.109
Aqeous LC	250	0.226	1.064
Methanolic HC	500	0.210	1.086
Methanolic LC	250	0.198	1.038
Pet. Ether HC	500	0.230	0.998
Pet. Ether LC	250	0.207	0.924

NB: Normal range source: Cheesbrough (2003)
Total bilirubin is up to= 0.25 mg/dl, Direct bilirubin is up to= 1.10 mg/dl
KEY: HC= High Concentration, LC= Low concentration

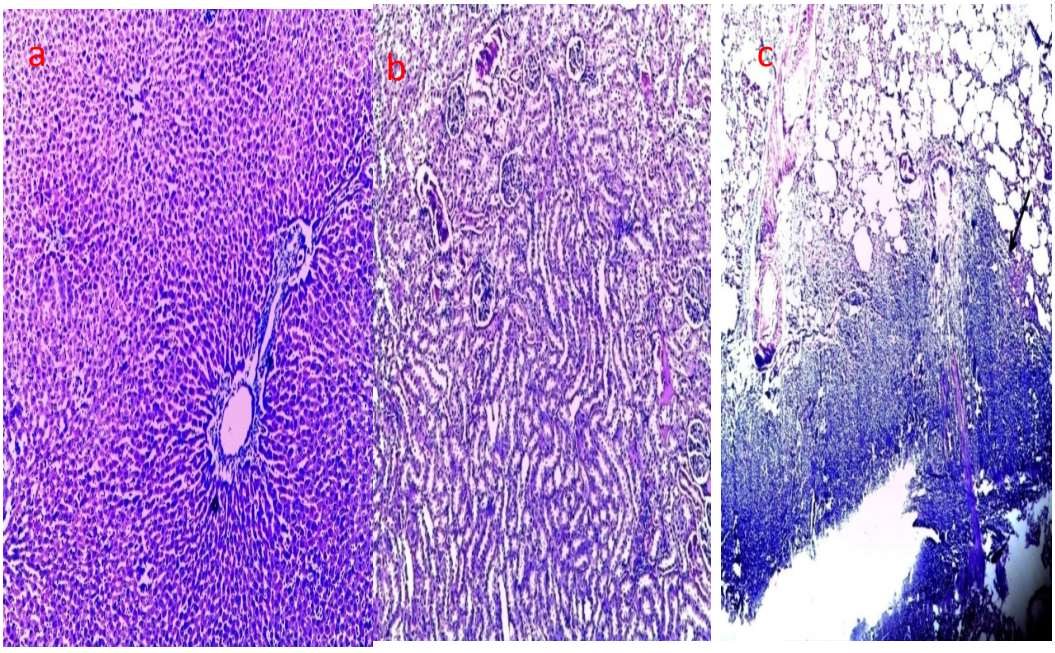

Plate I: Histological images of control tissues of rats treated with Distilled water extract of *Diospyros mespiliformis* (a. Liver b. Kidney c. Lungs

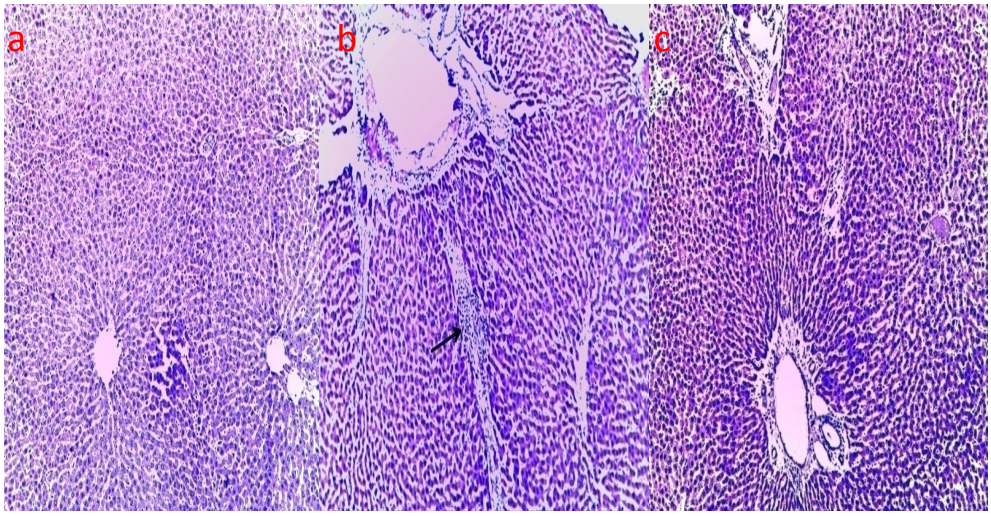

Plate II: Histological images of Liver Tissues of rats treated with different extract of *Diospyrosmespiliformis* (a. Aqueous extract b. Methanol extract c. Petroleum Ether extract)

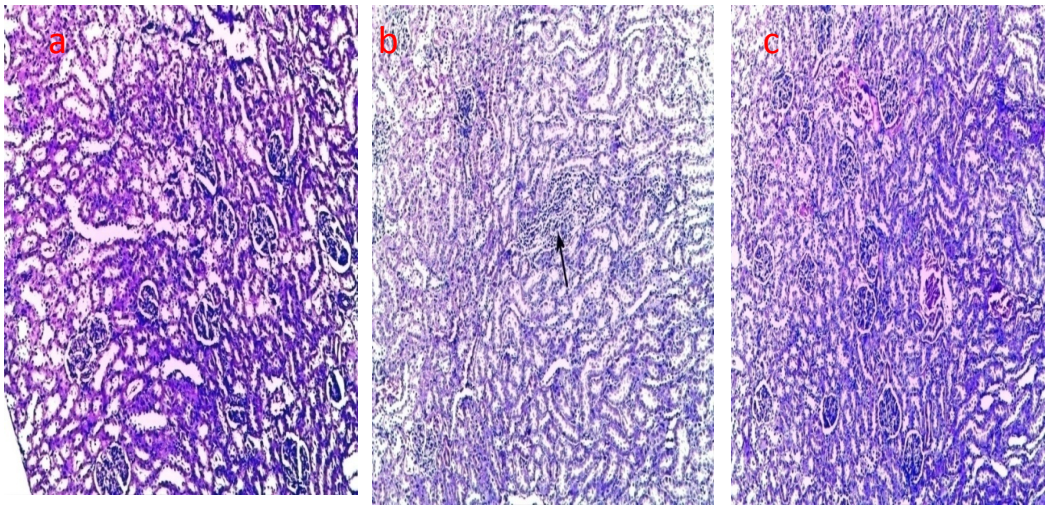

Plate III: Histological images of Kidney Tissues of rats treated with different extract of *Diospyrosmesipiliformis* (a. Aqueous extract b. Methanol extract c. Petroleum Ether extract)

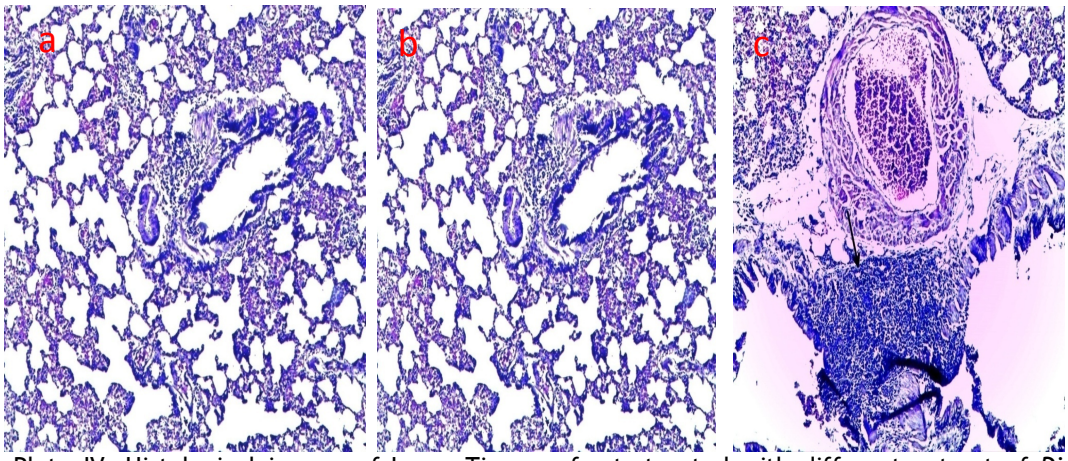

Plate IV: Histological images of Lungs Tissues of rats treated with different extract of *Diospyros mespiliformis* (a. Aqueous extract b. Methanol extract c. Petroleum Ether extract)

DISCUSSION

Results of phytochemical screening revealed six phytochemical constituents (flavonoids, alkaloids, balsams tannins, steroids and cardiac glycosides). Ebbo *et al*. (2014) have demonstrated the presence of tannins, steroids, cardiac glycosides, alkaloids and flavonoids in the stem bark extract of the plant. The high extraction property of methanol can be related to its amphiphilic property and high polarity index value (5.1) which makes it an ideal solvent for the extraction of polar and non-polar compounds.

Mahmood *et al*. (1997) reported the anti gp120-CD4 binding activity of flavonoids extracted from *Cuscuta reflexa*. Similarly, Williamson *et al*. (2006) highlighted the anti gp120-CD4 binding potentials of flavonoid, epigallocatechingallate (EGCG), extracted from green tea. In addition, Ashok *et al*. (1995) reported the anti gp120-CD4 binding activity of novel alkaloids extracted from sponge *Batzella* sp. Gen-ichiro *et al*. (2004) also reported that tannins possessed inhibition property against HIV-1 gp120-CD4 cell binding. Furthermore, Bridgette *et al*. (2016) reported that tannins possess gp120-CD4 binding inhibition property. Similarly, Gen-ichiro *et al*. (2004) reported that all tannins appear to inhibit virus-cell interactions. Thus, in spite of their anti-RT activity, the mechanism by which tannins inhibit HIV may not be associated with this enzyme. The anti gp120-CD4 binding property exhibited by the extracts were higher at lower concentrations, this is one of the important qualities required by an ideal anti-microbial agent. Furthermore, Rege *et al*. (2010) reported the anti GP120-CD4 binding potential of medicinal plants namely; *Ocimum sanctum, Withania somnifera, Tinospora cordifolia, Avicenia, and Rhizophora mucronata,* and most of the plants tested exert their anti HIV activity via multiple mechanisms of action, viz. interference with the gp120/CD4 interaction and inhibition of viral RT. Hashim *et al*. (2015), have elucidated the anti HIV-1 gp120-CD4 binding activity of *Leptademia hastate* and *Vernoniaamygdalina*. Moreover, In Woo Park *et al*. (2009) had reporte d that Euphorbiaceae, Trigonostemaxyphophyll oides (TXE) and Dipterocarpaceae, Vaticaastrot richa (VAD) inhibit HIV-1 replication likely by blocking HIV-1 interaction with target cells, i.e., the interaction between gp120 and CD4/CCR5 or gp120 and CD4/CXCR4 and suggested the potential of developing these two extracts to be HIV-1 entry inhibitors.

Kurt and Dominique (2005), worked on anti-HIV agents targeting the interaction of gp120 with the cellular CD4 receptor, where the cyclotriazadisulfonamide compounds tested for their anti gp120-CD4 binding potential using the similar gp120 capture ELISA Kit but different brand, and this was also found to have inhibitory potential for HIV 1 gp120-CD4 binding.

Results of sub-acute toxicity of the test rats showed no case of death, weight loss, hair loss, diarrhea, or weaknesses from the test rats at the end of the 28 days period. This indicated that the extracts were not toxic to the treated animals.

Histological analysis conducted on the organs (liver, kidney, and lung) of the test rats shows no signs of inflammations in the rats treated with all the extracts.

Biochemical analysis of the test rats' sera showed significant elevation ($p<0.05$) of serum AST and ALP obtained in mice treated with the extracts. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indices for identifying inflammation and necrosis of the liver (Tilkian *et al.,* 1979). The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal and cardiac muscles, pancrease and kidney. ALT measurements are more liver specific than the AST and its activity is usually greater than AST activity at early or acute hepatocelluar disease (Whitby *et al.,* 1989). AST on the other hand tend to be released more than the ALT in chronic liver diseases such as cirrhosis (Whitby *et al.,* 1989). A normal ALT in the presence of elevated activities of AST and lactate dehydrogenase rules out the hepatic origin of the enzyme. In this study, ALT levels are normal but there was no significant changes ($p<0.05$) in the AST levels. The observed changes in AST were due to administration of 250-500 mg kg^{-1} body weight of the extracts to the experimental animals compared to the control group.

The activity of Alkaline phosphatase (ALP) is increased in many clinical states; the most important being bone and liver diseases. Accordingly, serum ALP is a useful diagnostic, screening and follow-up tool of cholestatichepatobiliary lesions and osteoblastic bone diseases (Wolf, 1978). Cholestasis is the main, if not the only liver disease responsible for increased plasma alkaline phosphatase activity. Thus, a normal alkaline phosphatase activity, in

the presence of abnormal levels of other liver function parameters, may be suggestive of liver pathology other than obstruction (Tilkian *et al.,* 1979). In the current research however, the enzyme activity of the animals on different doses differs significantly ($p<0.01$). This observation, vis-à-vis other clinical pictures ruled out the possibility of liver obstruction.

The effect of extracts on serum albumin level showed increased albumin and globulin values at 500 mg/kg methanol extract. Albumin is the most abundant of the plasma proteins with the physiological role of maintenance of osmotic pressure, transportation of both endogenous and exogenous substances and serving as protein reserve. The ability of the liver to synthesize albumin is diminished if the synthetic function of the organ is affected (Whitby *et al.,* 1989). While the serum total protein and globulin are within the normal range as in the current study, the assay of serum total protein alone may not portray the true picture of the metabolic state of the individual. This is because the concentration of the individual proteins, do not rise or fall in parallel with one another (Whitby *et al.,* 1989). Increased plasma total protein concentration may be due to dehydration, and increased plasma immunoglobulin concentration may be due to infection. In the current study the serum protein profiles were not significantly different between the animals on different doses of the extract for the toxicity tests. These results demonstrate the fact that the synthetic function of the liver of the animal exposed to oral sub-chronic doses is not affected. Additionally, there is no sign of infection as neither the globulin levels nor the A:G ratio of the animals treated with the extract doses were significantly ($p>0.05$) affected.

Results of this study also showed that there were no significant changes in the bilirubin levels of the animals treated with the extract doses of the stem bark extract of *Diospyros mespiliformis*. Consequently, it may be stated that the excretory function of the liver in the rats is not affected significantly as a result of the administration of the oral extract doses. Bilirubin is a useful index of the excretory function of the liver, in addition to its being a useful tool in the assessment of haemolytic anaemia.

Kidney function is affected by a number of factors, which may ultimately result in its failure. Causes of kidney failure include destruction of the tubules in the kidney by drugs, including phytochemicals. As a result, the two main functions of the kidney: the glomerular filtration and tubular re-absorption and secretion may be affected.

Serum Urea level is above the normal range (1.7-9.1 mmol/l) while the serum creatinine level is below the normal range (10-55 mg/dl) in this study.

Increased in the serum urea is an indication of renal failure. Though plasma urea concentration is less reliable than the creatinine as an index of GFR, by virtue of the fact that it diffuses back into the renal tubular cells and its plasma concentration is dependent on the state of the liver function and protein intake and oxidation (Tilkian *et al.,* 1979), estimation of the two complement each other in evaluating this function of the kidney.

One of the commonest causes of hyperuricaemia is gout, in which there are either tophi or acute arthritis (Tilkian *et al.,* 1979). Hyperuricaemia as a result of chronic renal failure can be ascertained by correlating uric acid level with urea and creatinine.

In this study the serum electrolytes levels showed sodium and potassium levels to be within the normal range (166-250 mEq/l) and (3.4-5.3 mEq/l) respectively. The levels of electrolytes in the blood are the outcome of fine regulatory mechanism of ionic charges and the osmotic balance. This homeostasis is achieved by an interplay involving the kidney, the lungs and endocrine system (Tilkian *et al.,* 1979). Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects the body against excessive fluid loss. Hypernatraemia though rare, may occur in dehydration and steroid hormone administration. Hyponatraemia, on the other hand is more common and may be due to chronic sodium losing nephropathy, loss of gastrointestinal secretion through diarrhoea or vomiting, loss from skin as a result of burns, loss from kidneys through the use of diuretics and metabolic loss through starvation or diabetic ketoacidosis. Potassium is the major intracellular cation with similar role to those of sodium. Hyperkalaemia is usually encountered frequently in renal failure, improper use of K^+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease and

massive tissue destruction (Eccles, 1993; Tilkian *et al.*, 1979). Excessive renal loss of potassium is associated with diuresis, renal loss as a result of potassium losing nephropathy or as a result of renal tubular acidosis. Other causes of hypokalaemia include excessive loss without adequate replacement as in chronic diarrhoea, malabsorption syndrome, perspiration and chronic fever, chronic stress, poor dietary habit, Cushing's syndrome, hyperaldosteronism, liver disease with ascites, use of some drugs such as indomethacin, phenylbutazone and steroid hormone (Eccles, 1993; Tilkian *et al.*, 1979; Whitby *et al.*, 1989). Plasma bicarbonate ion concentration is increased in respiratory acidosis and metabolic alkalosis but decreased in respiratory alkalosis and metabolic acidosis ((Eccles, 1993; Tilkian *et al.*, 1979, Whitby *et al.*, 1989; Holmes, 1993).

In this study, the serum electrolytes of the animals treated with sub-chronic doses of the extract were not significantly different ($p>0.05$). This is an indication that the extract may not have any significant effects on the water, electrolyte and acid-base balance not animals' normal serum levels of electrolytes of animals treated with extract of *Momordica balsamina* have also been reported (Geidam *et al.*, 2004).

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CONCLUSION AND RECOMMENDATION

Six phytochemical constituents; flavonoids, alkaloids, tannins, cardiac glycosides, balsalms and steroids were detected, with methanol extract tested positive in all. The flavonoids, alkaloids and tannins were found to possess inhibition of gp120-CD4 binding interaction. The extracts were found to be well tolerated by the test animals. It is recommended that further research should be carried on the pure isolates of the extracts.

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