Assessment of HIV 1 GP120/CD4 Binding Inhibition Potential of *Solanum nigrum* Crude Fruit Extracts

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Abstract: Human immunodeficiency virus (HIV) infection is still contributing significantly in morbidities and mortalities in the world today, more especially in developing countries. The drugs normally use to treat the infection are costly, toxic, and less effective due to resistance by HIV. In view of that an assessment of gp120-CD4 binding inhibition potential of *Solanum nigrum* crude fruit extracts was conducted between June–December, 2018 using gp120-CD4 capture ELISA kits. Aqueous, methanol, and petroleum ether extracts were prepared at 1000, 500 and 250 µg/ml and tested for gp120-CD4 binding inhibition. Sub-acute toxicity assay was done using albino rats; biochemical parameters including alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as well as bilirubin for liver and urea, electrolytes and creatinine for kidney functions were evaluated. Kidneys, liver and lungs of the animals were examined for histopathological damages. The results of the effect of crude aqueous, methanol and petroleum ether extracts of *Solanum nigrum* fruit against gp120-CD4 showed inhibition ranging from 1.3-17 % with 1000 µg/ml showing highest percentage of inhibition. There was no significant difference (P = 0.861) in terms of percentage inhibition between the three concentrations tested. Methanol extract demonstrated the highest percentage inhibition of gp120-CD4 bindings (17 %). No significant difference (P = 0.123) between the three extracts against gp-120-CD4 bindings was observed. The results of the sub-acute toxicity study have shown that, there were no physical changes in animals treated with 500 mg/kg of all the extracts. The result of liver function test revealed that, ALT, AST and ALP were within the normal range (12U/L) for both the high and low concentrations of the extracts including the control. Also result for total protein, albumin, globulin and albumin globulin ratio for the test albino rats and that of the control rat were found to be within the normal range 5.2-9,3.5-5, 2.0 – 3.5 g/dl and 0.8 – 2.0 for total protein, albumin, globulin and globulin ratio respectively. For serum electrolyte level, sodium and potassium ions for the various concentrations of the extracts tested and control were also found to be within normal range. The result of kidney function test revealed that, urea, creatinine and direct and total bilirubin of the rats tested and control were all found to be within normal range. In addition, the histopathology images, shows no remarkable inflammation in all the organs studied. In conclusion, fruit extracts of *Solanum nigrum* possessed some levels of HIV-1 gp120-CD4 binding inhibition potentials and the extracts were found to be non-toxic at 250 and 500mg/ kg body weight. It can be recommended that, the bioactive compounds should therefore be isolated and tested for gp120-CD4 binding inhibition activity.
Keywords: gp120-CD4 binding inhibition, *Solanum nigrum* crude extracts, Sub-acute toxicity test

INTRODUCTION

The HIV virion enters macrophages and CD4 T Cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the target cell membrane and the release of the HIV capsid into the cell (Chan and Klein, 1998; Wyatt and Klein, 1998).

The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine receptor binding domains of gp120 and allowing them to interact with the target chemokine receptor (Chan and Kim, 1998; Wyatt and Sodroski, 1998). This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

There are two related but distinct types of HIV: HIV-1 and HIV-2 (Fletcher *et al*., 2002). HIV-1 is the most pathogenic and causes over 99 % of HIV infections (Cos *et al*., 2004). HIV-2 is also known to cause AIDS but is much less prevalent; being present in fewer and isolated geographic locations such as West Africa, therefore, most research is done on HIV-1 (Klos *et al*., 2009). About 13 million people were infected with HIV worldwide in 1993 and the number has increased to about 21 million in 1996.

The number has increased to about 36.1 million in 2007 (WHO 2007) and slightly to 36.9 in 2014, with 3 million infected in Nigeria (WHO, 2014). About 36.7 million people were living with HIV around the world in 2016, and 19.5 million of them were receiving medicines to treat HIV, called antiretroviral therapy (ART). An estimated one million people died from AIDS-related illnesses in 2016. The number of HIV infected people has increased to 3.2 million in Nigeria. According to Nigeria HIV/AIDs indicator and impact survey (NAIIS), 1.9 million Nigeria live with the disease in 2019.

The World Health Organization (WHO) recommended that traditional healers be included in national responses to HIV/AIDS. As early as 1989, WHO had already voiced the need to evaluate ethno medicines for the management of HIV/AIDS. In this context, there is need or the systematic evaluation of the elements of traditional medicine, particularly medicinal plants and other natural products that might yield effective and affordable therapeutic agents (WHO, 2009). Plants are important source of drugs; especially in traditional medicine (Bako *et al.,* 2005).

Although there are reports on traditional uses of plants to treat various diseases, knowledge of herbal remedies used to manage HIV/AIDS are scanty, impressionistic and not well documented (Koyombo *et al.,* 2007; Chinsembu and Mutirua, 2008). Thus, it is important to search for novel anti-HIV agents that can be added to or replace the current arsenal of drugs against HIV (Klos *et al.,* 2009). *S. nigrum* is traditionally used in the treatment of some ailments in human and animals such as Newcastle disease in poultry; therefore, the study was aimed at assessing the gp120-CD4 binding inhibition potential of the crude extracts of *S. nigrum* fruits.

MATERIALS AND METHODS

Sourcing of Plant Materials

Solanum nigrum (Solanaceae) fruits were collected at Dawakin Kudu Local Government Area, Kano State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology, Bayero University, Kano and was given Herbarium Accession Number BUKHAN 393.

Extraction of Phytochemical Constituents

The phytochemical constituents *S. nigrum* was extracted using soxhlet extraction technique as described by Tariq *et al.* (2011).

Preparation of Aqueous Fruit Extracts

Solanum nigrum fruits were washed, shade dried and finely powdered. The powder 50g, was suspended in 500 ml of sterile distilled water as described by Tariq *et al*. (2011).

Phytochemical Analysis of the Extracts

The extracts were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, cardiac glycosides and reducing sugars as stated Adetuyi and Popoola (2001); Salehi *et al.*(2018).

Gp 120 Binding Inhibition Assay as described by Rege *et al.* (2009)

Components A (plate holder with solid phase capture ligand CD4). B (Wash buffer), C (blocking/diluents) was brought to room temperature, while the wash buffer (component B) was diluted 1:10 with deionizer water. Then, the plate was removed from shielded bag using scissors. Subsequently, two hundred micro liters (200 µl) of wash buffer was added to each well and was allowed to stand at room temperature until ready for the next step. Equally several dilutions of HIV gp120 (component D) was made in comparable tubes and label accordingly.

Preparation of the Test Concentrations and Screening for Anti gp120-CD4 Binding Activity as described by Rege *et al.* (2009)

Three (3) different concentrations (1000 µg/µl, 500µg/µl, and 250µg/µl) of the plant extracts were prepared in three (3) separate test tubes using diluents buffer (component C) and labeled accordingly. The content of the wells was dumped and pad dried to remove the wash solution and placed on a grid.

Equally the grid was labeled 1 – 3 extracts tested (1=aqueous fruit extract 2 = methanol fruit extract 3=petroleum ether fruit extract) while the grid was label A-H vertically for the control and different concentration of the test extracts (A and B are for the control, C and D for the 1000 µg/µl in duplicate, E and F for the 500 µg/µl in duplicate, G and H for the 250 µg/µl in duplicate).

One hundred micro liters (100 µl) of Gp120 positive reference was mixed with 100 µl each of the different concentrations of the test extracts and the control in a separate sterile micotitre plate and allowed to stand for ten (10) minutes. These were subsequently dispensed into the appropriate CD4 coated microtitre wells as labeled. The plate was covered and allowed to stand at room temperature for an hour. The contents of the wells were dumped and washed three times with wash buffer (300µl/well), the wells were pad dried.

Detector reagent (component E) was diluted 1:100 in diluents buffer (component C) and 100 µl of the detector reagent was added to each of the well and allowed to stand at room temperature for one hour. Subsequently the wells were washed three times using washing buffer (component B) and pad dried. One hundred micro liter (100 µl) of 3.3', 5.5'-Tetramethylbenzidine (TMB) substrate (component F) was added to each of the wells. Blue color developed within 10 minutes.

Color development was stopped by adding 100 µl of stop solution (Component G) to each well. The plate was read at 450 nm using ELISA reader within 5 minutes. The set up was duplicated, so that average values was taken. Percentage inhibition was calculated using formula: (Rege *et al.,* 2010).

$$Percentage\ (\%) inhibition = \frac{Optical\ Density\ of\ the\ sample}{Optical\ Density\ of\ the\ control}\ x\ 100$$

Sub-Acute Toxicity Study as described by Adeyomo and Makinde (2013)

Three groups of four animals (Swiss albino rats) were set up with each group horizontally representing three different labeled as aqueous, methanolic and pet-ether extracts. In each group, 2 animals were administered with 250mg/kg and the other 2 animals were given 500mg/kg extracts daily for 4 weeks. A control group of 2 animals that were only given 1ml of distilled water for the same period was set up. The body weight changes were monitored throughout the experimental period on weekly basis, while the animals were monitored for manifestation of toxicity and mortality.

Biochemical Analyses

Liver Function Tests

The following tests were conducted to investigate derangements in the liver function of animals used for sub-chronic toxicity studies: Serum total bilirubin, serum total protein and albumin. Serum globulin level was calculated as the difference between serum total proteins and Albumin. Albumin/Globulin (A/G) ratio was estimated from the values obtained for the albumin and globulin. Serum AST and ALT activities were determined using Reitman-Frankel (1957) method (Cheesbrough, 2000). Alkaline phosphatase activity was estimated in the serum by the nitrophenyl phosphate method of Bessey, (Cheesbrough, 2000).

Renal Function Tests

Serum createnine, urea, sodium and potassium ions were evaluated as described by Reitman *et al.* (2007); Cheesbrough (2000).

Histopathological Examination as described by Suryavathi *et al.* (2005)

After the animals were sacrificed, the abdomen of each was cut opened and the liver, kidneys and lungs were removed for biopsies. The organs were fixed with 10 % formal saline, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. Sections of the organs were stained with haemotoxylin and eosin. And observed under leica DM 750 microscope and photographed with leica ICC 50 HD Camera.

Statistical Analysis

The result was analyzed using Two-Way Analysis of Variance (ANOVA). The level of significance used was $P < 0.05$.

RESULTS

Phytochemical Physical Characteristics of *Solanum nigrum* Fruit Extract

The physical characteristics of *Solanum nigrum* fruit extracts and the results are shown in Table 1, in which the color ranges from chocolate, yellow to brown, while the texture was gummy and the pH ranges from 6.1 – 6.8 (Table 1).

The results of qualitative phytochemicals analyses of Aqueous, Methanol and petroleum ether fruit extracts revealed the presence of some phytochemical components such as saponins, flavonoids, steroids, and polysterols (Table 2).

Anti-binding Effect of the Extract against gp120 – CD4

The results of the activity of crude aqueous, methanol and petroleum ether *Solanum nigrum* fruit extracts against gp120-CD4 (Table 3) showed inhibition at various concentration ranging from 1.3 – 17 %. The highest inhibition was 17 %, at 1000 µg/ml of methanol followed by 15.3 %, and 14.3 % in both 1000 and 500 µg/ml of petroleum ether and methanol respectively (Table 3).

Result of Sub-Acute Toxicity of Fruit Extract of *Solanum nigrum*

Results showed that, oral administration of aqueous, methanol and petroleum ether of *Solanum nigrum* of 250 and 500mg/kg did not produce any sign of sub-acute toxicity (Such as, hair loss, weight loss, etc.) or instant death in any of rat group tested (Table 4).

Table 1: Physical Characteristics of the Fruit Extract of *Solanum nigrum*

Extracts	Color	Texture	Odor	Solvent	PH
Aqueous extract	Yellow	Oily	Odorless	DMSO	6.8
Methanol extracts	Chocolate	Oily	Odorless	DMSO	6.5
Pet. ether extracts	Brown	Oily	Odorless	DMSO	6.1

Table-2 Qualitative Phytochemical Analysis of the Aqueous, Methanol and Petroleum ether extract of *Solanum nigrum.*

Photochemical components	SNF. A Ex.	SNF. M. Ex.	SNF. P. Ex
Alkaloid	-	-	-
Saponin	+	+	+
Tannins	-	-	-
Flavanoids	+	+	+
Steroids	+	+	+
Trepenoids	-	-	-
Phenols	-	-	-
Antraquinone	-	-	-
Cardialglycoside	-	-	-
Phytosterol	+	+	+
Antthocyanin	-	-	-

Key: - + = positive, - = not detected

SNF = *Solanum nigrum* fruit, AE_X = Aqueous Extract, ME_X= Methanol extract, PE_X= Petroleum Ether extract

Table 3: Mean Optical Density of Fruit Extracts of *Solanum nigrum* against HIV1 GP120-CD4 Binding Inhibition at Various Concentrations in $\mu g/ml$

Extract	Concentration ($\mu g/ml$)/% Inhibition		
	1000	500	250
Methanol	17	14	10
Aqueous	1.3	1.3	10
Petroleum ether	15.3	12.1	9.6
Control	100	100	100

P-value - 0.003021

Table 4. Mean Percentage Weight Gain and Physical Effect of *Solanum nigrum* on Albino Rats

Extract	Conc. Of the extract Kg/BW	Initial Wt. (g)	Final Wt. (g)	Wet gain	% Wet gain	Hair loss	Diarrhea
Aqueous	250	102	105.5	3.5	3.4	N	N
	500	100.5	104.5	4.0	3.98	N	N
Methanol	250	100.5	107	6.5	6.47	N	N
	500	104.5	107	5.5	5.42	N	N
Petroleum ether	250	98.5	103.5	5.0	5.08	N	N
	500	99	103.5	4.5	4.55	N	N
Control	NIL	99.6	103.6		4.02	N	N

KEY: N = No

Table 5: Liver Enzymes Activities (U/l) of Rats Treated with Sub-Acute Oral Doses of Aqueous Fruit Extract of *Solanum nigrum*

Extract	Dose (mg/kg)	Parameter (Activity u/l)		
		AST	ALT	ALP
Control	0	12	12	8.0
AHC	500	8.0	8.0	13
ALC	250	12	8.0	13
MHC	500	12	12	8.0
MLC	250	12	4.0	13
PHC	500	8.0	12	8.0
PLC	250	12	8.0	8.0

KEY: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase

NB: Normal Range

AST = Up to 12U/I, ALT = Up to 12U/I, ALP = Up to 12U/I,

AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Adopted from Cheesbrough (2003)

Biochemical Parameters for Liver Marker Enzymes

Liver marker enzymes of AST, ALT and ALP. The AST, ALT and ALP are all found to be within the normal range (Table 5).

Serum Electrolyte Parameters

Results of serum electrolytes parameters (sodium ion, potassium ion and bicarbonate ions) were all within normal ranges for both high and low concentration of aqueous, methanol and petroleum ether extracts (Table 6).

Serum Protein Level

Results of serum protein level, Albumin and globulin of rats treated with *Solanum nigrum* crude fruit extracts were found to be within the normal acceptable range (Table 7).

Creatinine and Urea Level

The serum creatinine and urea concentrations were found to be within the normal range in all the various concentrations of the extracts tested including the control (Table 8).

Total and Direct Bilirubin of Serum

Results of total and direct bilirubin of the serum of the rats treated with high and low concentration of aqueous, methanol and petroleum ether fruit extract of *Solanum nigrum* and control group were within the normal range (Table 9).

Table 6: Serum Electrolytes Level of Rat Treated with Fruit Extract of *Solanum nigrum*

Extract	Dose (mg/kg)	Parameter (Activity u/l)		
		Na	K	HCO_3^-
Control	0	135.245	4.00	21.05
AHC	500	156.275	3.539	22.83
ALC	250	146.761	3.644	20.90
MHC	500	146.471	3.604	21.05
MLC	250	146.961	3.476	20.79
PHC	500	149.509	4.16	21.66
PLC	250	135.049	3.476	21.86

Key: Na^+ = Sodium ions, K^+ = Potassium ions, HCO_3^- = Hydrogen trioxocarbonate

NB: Normal Range (mEq/L)

Na^+=135-155

K^+=3.4-5.3

HCO_3^-=20-32

AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Adopted from Cheesbrough (2003)

Table 7: Serum Protein Level of Rat Treated with Fruit Extract of *Solanum nigrum*

Extract	Dose mg/kg	Conc. of PARAMETER			
		Total protein	Albumin	Globulin	A:G
Control	Water	5.477	4.418	2.574	1.716
AHC	500	5.739	4.405	2.666	1.652
ALC	250	5.392	4.378	2.986	1.466
MHC	500	6.221	4.554	3.333	1.366
MLC	250	5.729	4.108	3.379	1.216
PHC	500	5.477	4.932	3.455	1.427
PLC	250	5.050	5.121	3.071	1.668

NB=Normal Range, Total protein=5.2-9.1g/dl, Albumin= 3.5-5.0g/dl, Globulin = 2.0 – 3.5g/dl, A: G = 0.8 – 2.0

Adopted from Cheesbrough (2003)

KEY: Water= Control, LC=Low concentration, HC=High concentration, AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Table 8: Some Kidney Function Parameter of Rat Treated with *Solanum nigrum* Fruit Extract

Extract	Dose mg/kg	PARMETER	
		Urea(mmol/L)	Creatinine(mg/dl)
Control	Water	8.035	3.290
AHC	500	3.416	1.806
ALC	250	4.027	8.452
MHC	500	7.081	7.161
MLC	250	6.985	1.161
PHC	500	1.928	3.290
PLC	250	5.878	3.935

KEY: Water = Control, LC = Low concentration, HC = High concentration, **NB**: Normal Range, Urea=1.7-9.1mmol/L, Creatinine=Up to 20mg/dl
Adopted from Cheesbrough (2003)
AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Table 9: Serum Bilirubin Level of Rats Treated with *Solanum nigrum* Fruit Extract

	Dose	Concentration of parameters (mg/dL)	
Extract	mg/kg	Total bilirubin	Direct bilirubin
Control	0	0.096	0.147
AHC	500	0.199	0.171
ALC	250	0.158	0.210
MHC	500	0.144	0.189
MLC	250	0.201	0.222
P HC	500	0.174	0.172
PLC	250	0.177	0.166

KEYS: AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Histopathological Examination

The histopathological sections of the liver, lungs and kidneys of the rats treated with low and high concentrations of the plant extracts showed unremarkable tissue damages (Plates I, II, III, and IV). The source of the liver kidney and lungs treated with high and low concentrations of extract aqueous methanolic and pet ether extract revealed normal size, shape and appearance (Plate 1 to 4).

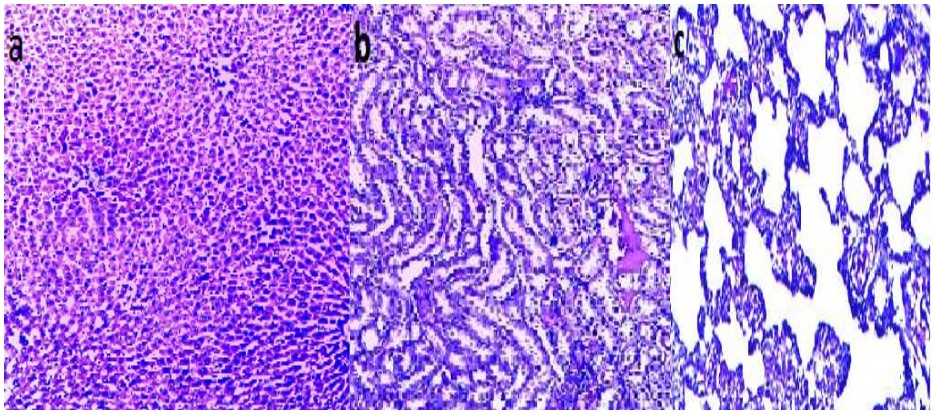

Plate I: Histopathological images of control rats (a. Liver b. Kidney c. Lungs)

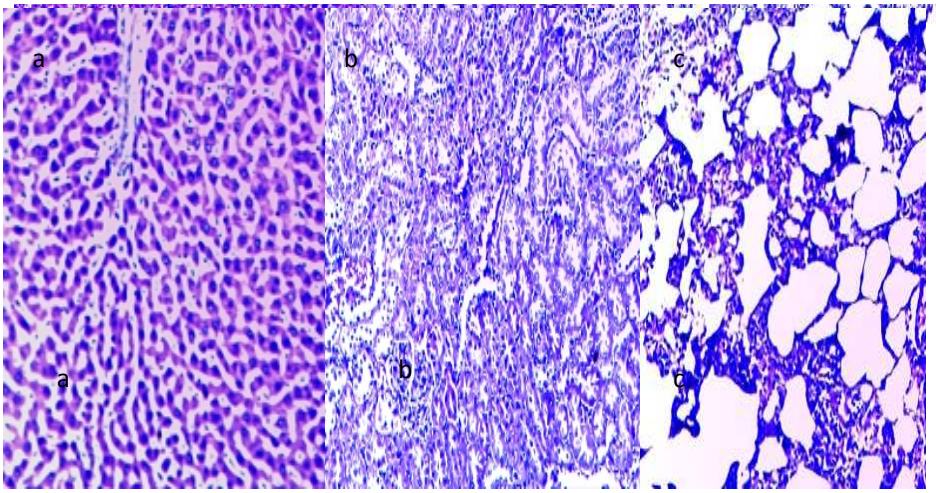

Plate II: Histopathological images of rats treated with aqueous extract of *Solanum nigrum* fruit (a. Liver b. Kidney c. Lungs)

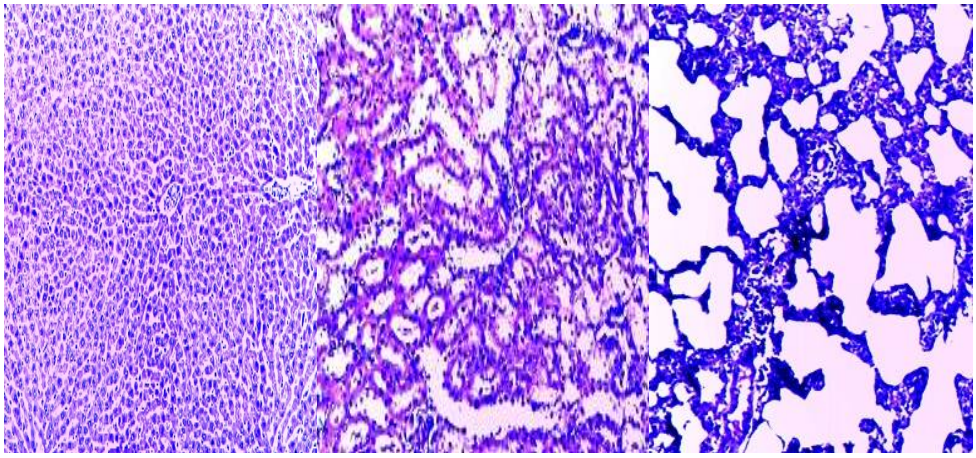

Plate III: Histopathological images of rats treated with Methanolic extract of *Solanum nigrum* fruit (a. Liver b. Kidney c. Lungs)

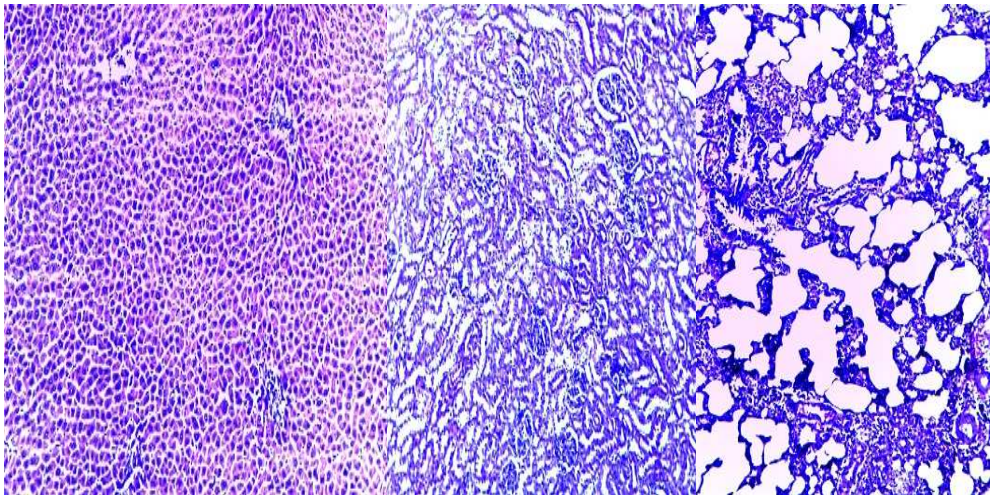

Plate IV: Histopathological images of rats treated with Petroleum extract of *Solanum nigrum* fruit (a. Liver b. Kidney c. Lungs)

DISCUSSION

The phytochemical screening of *Solanum nigrum* fruit extracts revealed the presence of saponins, steroids, flavonoids, and polysterols. This phytochemical study is in agreement with the work of Ahmad *et al.,* (2005) on the evaluation of the activities of *Solanum nigrum* fruit extracts which reported that, *Solanum nigrum* fruit extracts contains alkaloids, steroids, saponins, and phenols. Mature fruits are low in alkaloid contents on food chemical toxic. The compounds that blocked HIV-1 infection were flavonoids and anthocyanidins (Ryan *et al.,* 2009). Several chemical compounds were found to interfere with HIV entry into cells. BanLec, a jacalin-related lectin that binds to glycosylated viral envelopes blocked HIV-1 entry into cells (Swanson *et al*., 2010); cyanovirin, an 11 KDa protein isolated from *Nostoc ellipsosporum*, targeted gp120 proteins and blocked fusion of HIV-1 to lymphocyte membranes (Gustafson *et al*., 1992) glycoprotein complexes from *Ganoderma* mushrooms inhibited HIV-1 gp120 binding to CD4 cells (Lindequist *et al*., 2005); a code-named compound, PJ-S21, from *Punica granatum* inhibited the binding of gp120 to cells expressing CXCR4 receptors (Neurath *et al*., 2004). Other active constituents included: diterpene lactones (Calabrese, 2002) and a coumarin named wedelolactone (Yao *et al*., 2002) inhibited cell-to-cell transmission of HIV-1; prostratin, a 12-deoxyphorbol, inhibited HIV-1 entry intolymphocytes (Park *et al*., 2009); and rosmarinic acid isolated from *Melissa officinalis* inhibited fusion of HIV-1 to cells (Geuenich *et al*., 2008).

The oral administration of aqueous, methanol and petroleum ether of *Solanum nigrum* of 5000mg/kg did not produce any sign of sub-acute toxicity or instant death in any of rat group tested. This suggested that, the extract has a low toxicity of 5000mg/kg body weight. This work was in agreement with the work of on the evaluation of *Solanum nigrum* fruit extract. They reported that, *Solanum nigrum* fruit extract did not produce any mortality even at the dose of 1500 mg/kg. All the doses (5, 50 and 300 mg/kg,) of *Solanum nigrum* were thus found to be non-toxic. Any substances with a toxicity ranged between 2000-5000 mg/kg body weight given orally could be considered of low toxicity.

In the assessment of liver damage, certain biomarkers of hepatotoxicity are measured and such biomarkers are enzyme levels such as AST, ALT, ALP, total bilirubin and GGT because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; thus, measurement of these enzymes in serum gives an

indication of the health status of the liver. High levels of AST indicate liver damage, as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyzes the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. It is known that an increase in the enzymatic activity of ALT, AST and GGT in the serum directly reflects a major permeability or cell rupture, and thus ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Häussinger, 2011). The serum marker enzymes such as AST, ALT, ALP and total bilirubin are all within the normal range. And this is in agreement with the research by Ryan, (2009) on the "effect of dried fruit of *Solanum nigrum* LINN against CCl_4-Induced Hepatic Damage in Rats" revealed that, the rat treated with the extract along with toxicant showed sign of protection against these toxicant to a considerable extent as evident from the formation of normal hepatic cards and absence of necrosis and vacuoles. Also decrease in serum bilirubin after treatment with the extract in the liver damage indicates the effectiveness of the extract in normal functional status of the liver, (Ryan *et al.,* 2009).

Histological analysis of the liver treated with *Solanum nigrum* fruit extract at a concentration of 500mg/kg showed normal appearance of hepatic lobules and hepatic cells in all the extract tested. And that of kidney treated with the same concentration of the plant showed normal renal corpuscles, renal tubules, glomeruli and bowman's capsule in all the extract tested. And on the other hands, lung examination showed normal bronchi and bronchioles with normal parenchymal cell in all the extract tested. This histopathological examination of rats' organ treated with different extract of *Solanum nigrum* was in agreement with the work of Arya *et al.,* (2017).

CONCLUSION AND RECOMMENDATION

It can be concluded that extracts of *Solanum nigrum* possess some gp120-CD4 binding inhibition potentials with methanolic extracts having higher binding inhibition potential. Moreover, the plant extracts were found to be non-toxic to the animals used for the study. It is recommended that pure compounds should be isolated and tested for anti-gp120-CD4 binding activity *in vitro*.

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