African Journal of
Biochemistry and Molecular Biology Research

ISSN : 3434-6120

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v2i2.5183

Effect of Aqueous Extract of Achyranthes aspera Leaves on Antiretroviral Drug-Induced Hepatotoxicity in Rats

Isaac John Umaru[1], Mary Athanasius Udoh[2], Tensaba Andes Akafa[3], Joseph Oteng[4], Ingwu Joseph Akem[5], Ocheifa Mathew Ngbede[6], Ashaka Fidelis Utioukpan[7], Aboki Nwunuji Mijinyawa[8], Odok Endurance Akam[9], Ogholo Ogholo Ekup[10]

[1,3]Federal University Wukari, Taraba State, Nigeria; [2]University of Uyo, Uyo, Akwa Ibom. Nigeria; [4]Ghana Health Service, Public Health Department, Ghana; [5]Department of Environmental Health Abia State, Nigeria; [6]Federal Medical Centre, Makurdi, Benue State, Nigeria; [7]Taraba State College of Technology Takum, Taraba State, Nigeria; [8]College of Health Technology Calabar, Nigeria; [9]Open University Nigeria, Calabar, Nigeria
umaru.isaac@fuwukari.edu.ng

Article Info:

Submitted:	Revised:	Accepted:	Published:
Feb 15, 2025	Feb 28, 2025	Mar 12, 2025	Mar 17, 2025

Abstract

Achyranthes aspera leaves are believed to reverse drug resistance and increase the efficacy of current drugs. Achyranthes aspera leaves contain many secondary metabolites needed for the redressal of diseases and ailment. Thus, the ART-induced hepatotoxicity in rats. Methodology: Thirty (30) albino rats were divided into 5 groups of 6 each and treated as follows: Group A (no antiretroviral drugs, no extract); group B (antiretroviral drugs alone); group C (extract alone); group D (antiretroviral drug plus 40 mg/kg extract); group E (antiretroviral drug plus 80 mg/kg extract). All treatment lasted for twenty-eight days. Blood samples were collescted and serum ALT and AST determined using UV-spectrophotometer. The mean (± S.E.M) of data were calculated and further analysed for statistical significance using graph Pad Prism 5.0. Results: Mean serum ALT were 36.8 ± 20.24, 56.11 ± 6.12, 54.6 ± 24.18, 90.40 ± 11.14, 88.36 ± 13.10 and that of AST were 143.8 ± 20.24,

AJBMBR Journal is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License

208.4 ± 16.13, 60.0 ± 24.18, 164.40 ± 11.14, 52.36 ± 16.14 for groups A, B, C, D, and E respectively. There was a statistically significant difference between the mean values of serum AST for group B and those for group C. However, there was no statistically significant difference between the ALT values for the test and control groups of rats (p value >0.999) Also, there was no statistically significant difference between the mean values of AST for group B and those of groups A, D, E. Conclusions: This extract at a higher concentration should add to the treatment of HIV in synergy with antiviral drugs, However, the extract shown no significant reduction of serum ALT and AST in ART treated rats.

Keywords: Aqueous, Leaf, Extract, Achyranthes Aspera, Leaves, Antiretroviral, Drug, Induced, Hepatotoxicity, Rats

INTRODUCTION

Knowledge of herbs has been handed down from generation to generation for thousands of years [1]. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive [2]. In the recent past there has been a tremendous increase in the use of plant-based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. An upward trend has been observed in the research on herbals. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care [4]. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants [5]. In recent time there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day [6].

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. One of the many plants which

are being evaluated for their therapeutic efficacies is Achyranthes aspera which is commonly known as Latjeera (Hindi) & Rough Chaff tree (English). It is an erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides, on roadsides [7, 8, 9]. Although it has many medicinal properties, it is particularly used spermicidal [10], antipyretic [11] & as a cardiovascular agent [12].

Traditionally, the plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases [13]. For snake bites the ground root is given with water until the patient vomits and regains consciousness. Inhaling the fume of Achyranthes aspera mixed with Smilax ovalifolia roots is suggested to improve appetite and to cure various types of gastric disorders [14]. It is useful in haemorrhoids, leaves and seeds are emetic, hydrophobia, carminative, resolve swelling, digestive and expel phlegm. Ash of the plant is applied externally for ulcers and warts. The crushed leaves rubbed on aching back to cure strained back [15].

A fresh piece of root is used as tooth brush. Paste of the roots in water is used in ophthalmia and opacities of the cornea. Paste of fresh leaves is used for allaying pain from bite of wasps [16]. The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties [17, 18].

W. Shibeshi et al. [19] studied effects of methanolic extract of the leaves and reported for antifertility activities such as abortifacient, estrogenesity, pituitary weight, and ovarian hormone level and lipids profile in female rats. The abortifacient effect of the methanolic extract of the leaves of Achyranthes aspera was determined by counting the dead fetuses in vivo. Effect on estrogenesity was assessed by taking the ratio of the uterine weight to body weight. The ratio of the pituitary weight to body weight was also calculated. The effect of the extract on the level of ovarian hormones and lipid profile were evaluated using electrochemiluminescence immunoassay [19].

Hepatotoxicity, defined as a rise in either (a) alanine transferase (ALT) level more than three times of upper limit of normal (ULN), (b) alkaline phosphatase (ALP) level more

than twice ULN, or (c) total bilirubin level more than twice ULN when associated with increased ALT or ALP [20], is one of the most serious adverse effects of antiretroviral therapy (ART). This often results in discontinuation of treatment leading to worsening of the patient's condition [21]. Discontinuation of antiretroviral treatment worsens drug resistance which is also very common with these agents. It has also contributed to increased incidence of hospital admissions [22].

Though hepatotoxicity occurs with most antiretroviral regimen, it is more common with the older agents. In particular, studies show that protease inhibitors (PI) such as indinavir and ritonavir are more likely to produce liver toxicity compared to other agents [23]. Antiretroviral-induced hepatotoxicity occurs more in HIV patients co-infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) as treatment for HBV and HCV improved tolerance to antiretroviral drugs [24]. Also, heavy alcohol intake, female gender, old age, high CD4+ count and previous exposure to ART are important risk factors for antiretroviral induced hepatotoxicity [25, 26].

The prevalence of hepatotoxicity in HIV/AIDS patients starting new antiretroviral treatment ranges between 4.5% and 10% [27]. Moderate or severe hepatic injury is defined as 5-fold or 10-fold increase in plasma aminotransferases (AST, ALT) levels relative to pre-treatment baseline.

Mechanisms of ART-induced liver damage include direct hepatic injury, immune reconstitution, hypersensitivity reaction, and mitochondrial injury [28, 29,30,31].

There is documented evidence for the metabolism of antiretroviral drugs in rats. It has been documented that ritonavir, saquinavir, and efavirenz inhibited biliary excretion of taurocholic acid in human and rat hepatocytes [32]. It has also been demonstrated that efavirenz, abacavir, and lamivudine at dosages of 0.43 mg/kg, 0.43 mg/kg, and 0.21 mg/kg respectively independently elevated hepatic enzymes in albino rats after oral administrations for one week [33,34].

Certain therapeutic manipulations are reported to ameliorate ART-induce hepatic injury. In one study, it was found that procysteine, an intracellular glutathione stimulator, when combined with antiretrovirals reduced the toxicity of the latter [35]. Also, magnesium dietary supplementation has been found to suppress anti-retroviral-induced oxidative stress in rat hepatocytes [36]. However, these orthodox therapeutic interventions present their own challenges. For example, interferon-alpha induces elevation of aminotransferases

among other adverse effects [37]. There is, therefore, need to search for alternative agents such as herbal remedies that will ameliorate hepatotoxic effects of antiretroviral agents.

Some herbal remedies are reported to be hepatoprotective and have therefore found application in the management of liver disorders [38,39]. One of the medicinal plants credited with such hepatoprotective activity. This plant, also known Achyranthes, Species – Aspera belongs to the family Amaranthaceae. It is It is useful in haemorrhoids, leaves and seeds are emetic, hydrophobia, carminative, resolve swelling, digestive and expel phlegm. Ash of the plant is applied externally for ulcers and warts. The crushed leaves rubbed on aching back to cure strained back [40]. foods and also in the treatment of fever, diarrhea, paste of fresh leaves is used for allaying pain from bite of wasps [41]. The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties [42,43].

This study is justified by the fact that there is high incidence and prevalence of anti-retroviral induced hepatotoxicity. For instance, the prevalence of hepatotoxicity in HIV/AIDS patients starting new antiretroviral treatment ranges between 4.5% and 10% [44]. Hepatotoxicity, when moderate or severe, may warrant discontinuation of antiretroviral therapy with the attendant increases in hospital stay, cost of treatment, and morbidity/mortality. The disruption of therapy also encourages the emergence of resistant strains of HIV which increases the prevalence of HIV/AIDS and the resultant public health concerns.

The main objective of the study was to determine if the aqueous leaf extract of Achyranthes aspera has a protective effect on ART induced hepatotoxicity in rats. Specific objectives included:

1. To determine the effect of aqueous leaf extract of Achyranthes aspera on serum ALT and AST in rats.
2. To determine the effect of aqueous leaf extract of Achyranthes aspera on serum ALT and AST following ART-induced hepatotoxicity in rats.
3. To determine the effect of increasing doses of aqueous leaf extract of Achyranthes aspera on ALT and AST following ART-induced hepatotoxicity in rats.

MATERIALS AND METHODS

This study procedures were in accordance with guidelines for the care and use of laboratory animals [45].

Calculation of Sample Size

Sample size of 30 rats at 95% power to detect a difference between means of 2.5 at a significant level (alpha) of 0.05 (two tailed) was chosen using the special formula for the calculation of sample size for laboratory animals' experiments [45]:

$$N = 1+2C[s/d]^2 \ldots\ldots\ldots 1$$

Where, C = a constant (7.8) at 0.05 level of significance; s = 2.75 (standard deviation from a similar previous study) [29]; d = difference between means desired in present study.

Animal (Albino rats)

Thirty (30) rats of both sexes, 6-8 weeks old, were obtained from the animal house, HAUEMM, Federal housing Estate Bajabure Gerie. Adamawa state. Nigeria. The animals were certified healthy by a veterinarian. Each group of 6 rats was housed in a metal cage measuring 60cm x 45 cm x 30 cm and was allowed free access to animal feeds and clean drinking water. Left over feeds and water were discarded and the cages cleaned with chlorhexidine antiseptic solution every 12 hours. Artificial light was provided by fluorescent lamps (18 watts) and light-dark cycle of 12-12 hours maintained. The animals were maintained in this arrangement for two weeks for acclimatization.

Preparation of Plant Extracts

One kilogrammes of fresh leaves of Achyranthes aspera was collected, washed under running tap water, and air-dried at room temperature. Thereafter, the dried leaves were ground into fine powder and 50 grams of the powder was extracted with 500 ml of distilled water using the Soxhlet method [30]. The filtrate was obtained by solvent evaporation.

Experimental Procedure

The rats were randomly divided into 5 groups of 5 each. Thereafter, we treated the animals with the following drugs by gastric gavage for 28 days [45] as follows:

Group A: Each rat was given normal feed (no ART, no extract)

Group B: Each rat was given efavirenz (Ranbaxy, India) 8.6 mg/kg, abacavir (Ranbaxy, India) 8.6 mg/kg, and lamivudine (Ranbaxy, India) 4.3 mg/kg [46].

Group C: Each rat was given 80 mg/kg aqueous leaf extract of Achyranthes aspera.

Group D: Each rat was given efavirenz 8.6 mg/kg, abacavir 8.6 mg/kg, and lamivudine 4.3 mg/kg, plus 40 mg/kg aqueous leaf extract of Achyranthes aspera [47].

Group E: Each rat was given efavirenz 8.6 mg/kg, abacavir 8.6 mg/kg, and lamivudine 4.3 mg/kg, plus 80 mg/kg aqueous leaf extract of Achyranthes aspera.

A treatment chart was maintained. After 28 days, blood samples were collected from each rat for the estimation of serum ALT and AST.

Collection of Blood Samples

The rats were anaesthetized, one at a time, using intramuscular ketamine and diazepam 50 mg/kg and 5 mg/kg respectively [48]. Thereafter, blood samples were collected using the method described by Hoff [49]. Briefly, the skin over the jugular vein was cleaned with methylated spirit-soaked cotton wool and 1.0-2.0 mls of whole blood withdrawn through the jugular vein using a 25G hypodermic needle fitted unto a 2 ml syringe. The withdrawn blood samples were transferred gently into plain specimen bottles and allowed to clot naturally. Thereafter, the serum was separated into another specimen bottles using a micropipette and stored at 2-8°C until use.

Determination of Serum ALT and AST

We determined the serum ALT and AST using AST and ALT kits (Randox, United Kingdom) and UV-VIS spectrophotometer (Spectrum lab 752S, New Life Medical, England) based on the principle described by Reitman and Frankel [50].

Statistical Analysis

The mean values ± S.E.M. of AST and ALT were calculated. Then the data were tested for normality using D'Augostino and Pearson omnibus normality test. The data failed the D'Augostino and Pearson omnibus normality test. Therefore, values of serum ALT and AST obtained for the test and control groups of rats were tested for statistically significant differences using Mann Whitney test or Kruskal-Wallis test plus Dunn's multiple comparisons. There was no transformation of data. All the statistical tests were performed using Graph Pad Prism 6.0 and the results taken as statistically significant if the p value < 0.05.

RESULTS

Yield of the Extract Fifty (60) grams of the powdered leaves yielded 25 grams of extract giving a percentage yield of 47.

Serum Aminotransferases

The values of serum ALT and AST obtained were within the reference values. Contrary to expectations, these values did not change when increasing doses of the extract were added to the antiretroviral drugs.

The mean values (± S.E.M) of serum ALT are shown in Table 1. When subjected to further analysis, there was no statistically significant difference between the ALT values for the test and control groups of rats as shown in table. 1 (p value > 0.999).

The mean values (± S.E.M) of serum AST are shown in Table 2. Further analysis of the data revealed that there was a statistically significant difference between the mean values for group B (ART alone) and those for group C (extract alone) as shown in tables. 2 and 4 (p value = 0.016).

However, there was no statistically significant difference between the mean values of serum AST for group B (ART alone) and those of group A (no extract, no ART), group D (ART + 40 mg/kg extract), or group E (ART + 80 mg/kg extract). Also, increasing the dose of the extract from 40 mg/kg (group D) to 80 mg/kg (group E) did not significantly affect the level of serum AST as shown in Fig. 3 (p value = 0.659).

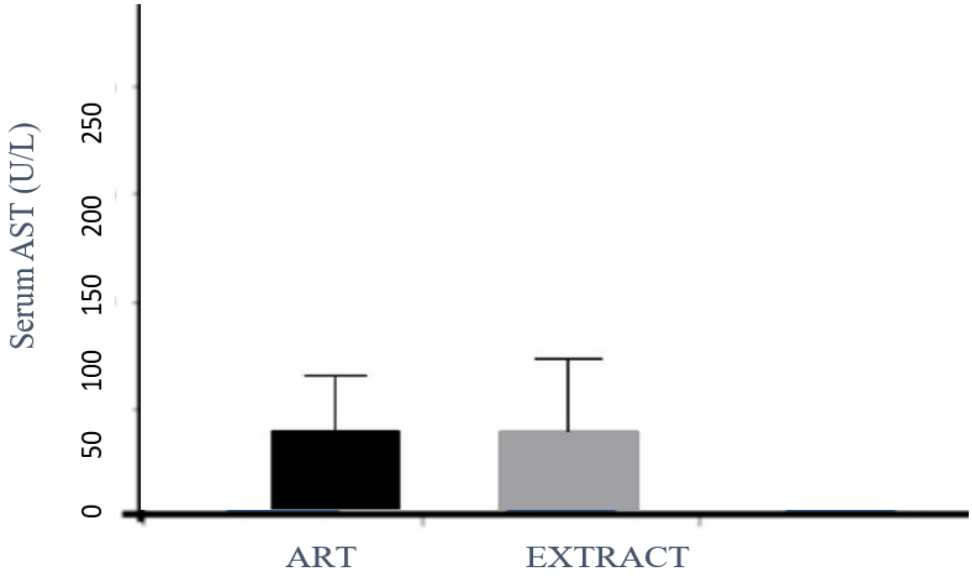


Figure 1: B vs C

Table 1: Serum alanine aminotransferase (ALT) values of rats treated with antiretroviral drugs (ART) alone (group B) compared to those of rats treated with aqueous leaf extracts of Achyranthes aspera alone (group C) using nonparametric Mann Whitney test.

	SERUM ASPARTATE AMINOTRANSFERASE (AST)	
	Table Analysed	**Data-1**
	Column B vs Column C	Extract alone vs ART alone
	Mann Whitney test	
	P-Value	>0.9999
	Extract or approximate P-value	Extract
	p-value summary	Ns
	Significantly different (P< 0.05)	No
	One- or two-tailed P-value?	Two-tailed
	Sum of ranks in Colum A, B	25, 20
	Mann-Whitey U	10
	Differences Between Medians	
	Median of Column A	58.00, n=6
	Median of Column B	54.60, n=4
	Difference Actual	-3.800
	Difference Hodges-Lehmann	-1.200

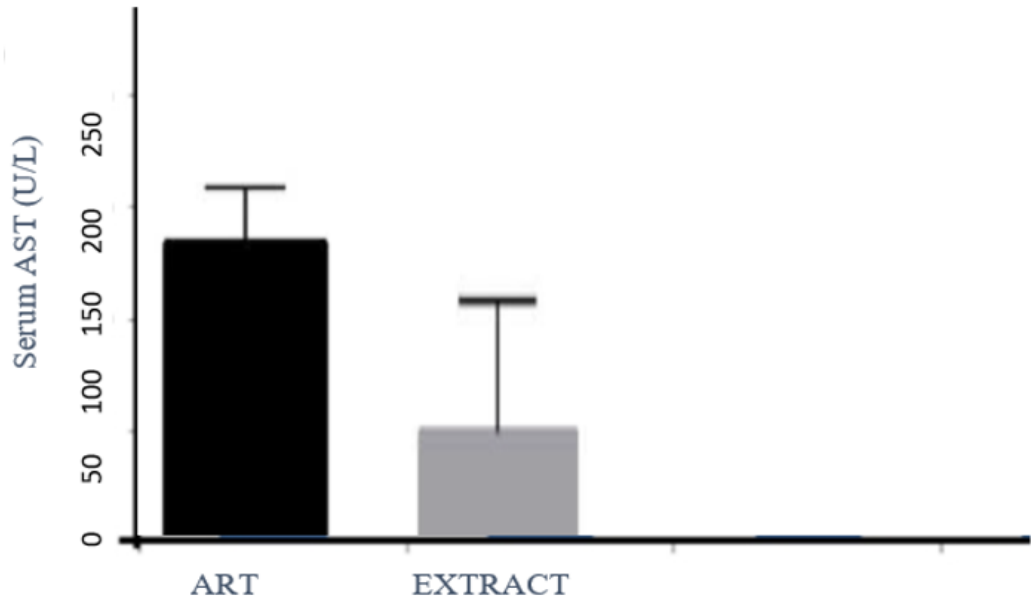


Figure 2: B vs C

Table 2: Serum aspartate aminotransferase (AST) values of rats treated with antiretroviral drugs (ART) alone (group B) compared to those of rats treated with aqueous leaf extracts of Achyranthes aspera alone (group C) using nonparametric Mann Whitney test

	SERUM ASPARTATE AMINOTRANSFERASE (AST)	
	Table Analysed	**Data-1**
	Column B vs Column C	Extract alone vs ART alone
	Mann Whitney test	
	P-Value	0.0160
	Extract or approximate P-value	Extract
	p-value summary	-
	Significantly different (P< 0.05)	yes
	One- or two-tailed P-value?	Two-tailed
	Sum of ranks in Colum A, B	38, 20
	Mann-Whitey U	0
	Differences Between Medians	
	Median of Column A	198.00, n=6
	Median of Column B	54.60, n=4
	Difference Actual	-166.8
	Difference Hodges-Lehmann	-154.0

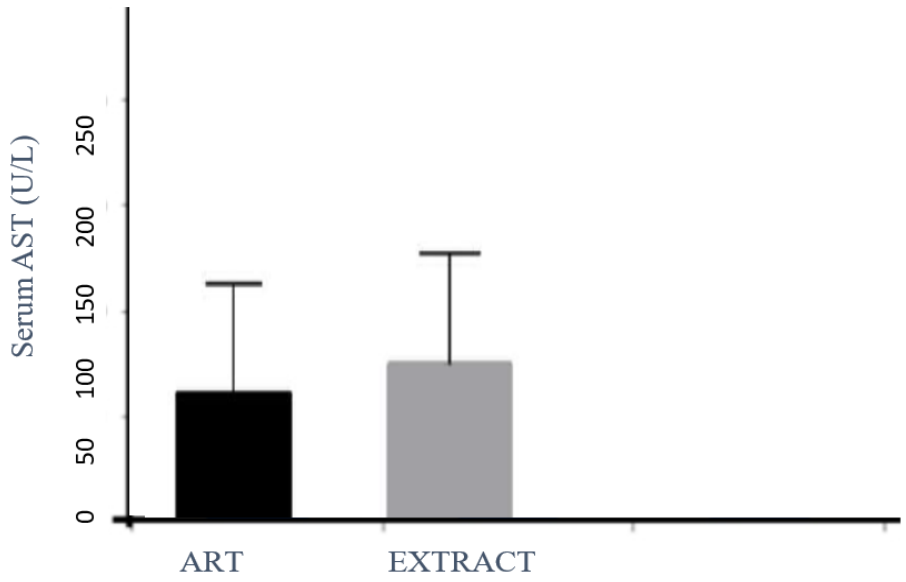


Figure 3: D vs E

Table 3: Serum aspartate aminotransferase (AST) values of rats treated with antiretroviral drugs (ART) plus 40 mg/kg aqueous leaf extracts of Achyranthes aspera (group D) compared to those of rats treated with (ART) plus 80 mg/kg aqueous leaf extracts of Achyranthes aspera (group E) using nonparametric Mann Whitney test

	SERUM ASPARTATE AMINOTRANSFERASE (AST)	
	Table Analysed	**Data-1**
	Column B vs Column A	ART + 80mg/Kg Extract vs ART 40mg/Kg Extract
	Mann Whitney test	
	P-Value	0.6572
	Extract or approximate P-value	Extract
	p-value summary	ns
	Significantly different (P< 0.05)	no
	One- or two-tailed P-value?	Two-tailed
	Sum of ranks in Colum A, B	16, 20
	Mann-Whitey U	7
	Differences Between Medians	
	Median of Column A	100.00, n=6
	Median of Column B	140.60, n=4
	Difference Actual	40.00
	Difference Hodges-Lehmann	26.80

Table 4: Serum Aspartate Aminotransferase (AST) Values of Rats Treated with Antiretroviral Drugs (ART) Alone (Group B)

SERUM ASPARTATE AMINOTRANSFERASE (AST) VALUES						
Subjects						
Number of Families	1					
Number of comparisons per Family	4					
Alpha	0,06					
Dunn's	Mean rank difference	Significant	Summary	B-		
Dunn's Multiple Comparisons Test	6.800	No	ns	A		No extract, no ART
	12.45	Yes	*	C		Extract alone
ART alone vs No Extract, no ART	8.450	No	ns	D		ART + 40mg/kg extract
ART alone vs Extract alone	5.300	No	ns	E		ART + 80mg/kg
ART alone vs ART + 40mg/kg						
ART alone vs ART + 80mg/kg						
Test Details	Mean rank 1	Mean rank 1	Mean rank 1	n 1	n 2	
ART alone vs No Extract, no ART	17.60	11.00	6.800	5	5	
ART alone vs Extract alone	17.60	5.300	12.45	5	4	
ART alone vs ART + 40mg/kg	17.60	9.800	8.350	5	4	
ART alone vs ART + 80mg/kg	17.60	13.00	5.200	5	4	

NB: Serum aspartate aminotransferase (AST) values of rats treated with antiretroviral drugs (ART) alone (Group B) compared with those treated with a combination of ART and extract of Achyranthes aspera using Kruskal-Wallis test followed by Dunn's multiple comparison test. [Group A (no extract, no ART), group C (extract alone), group D (ART + 40 mg/kg extract), group E (ART + 80 mg/kg extract)]

Table 5: Mean (± S.E.M) Serum Alanine Aminotransferase (ALT) Values (U/L) In the Test and Control Groups of Rats

Group A (A1-A5)	Group B (B1-B5)	Group C (C1-C4)	Group D (D1-D5)	Group E (E1-E4)
32.00	64.00	28.00	246.00	270.00
36.00	84.00	112.00	55.00	156.00
24.00	25.12	10.00	76.00	130.00
30.00	46.00	80.00	124.00	55.00
64.00	57.00	0.00	0.00	0.00
Mean				
36.8 ± 20.24	56.11 ± 6.12	54.6 ± 24.18	90.40 ± 11.14	88.36 ± 13.10

Table 6: Mean (± S.E.M) Serum Aspartate Aminotransferase (AST) Values (U/L) In the Test and Control Groups of Rats

Group A (A1-A5)	Group B (B1-B5)	Group C (C1-C4)	Group D (D1-D5)	Group E (E1-E4)
128.00	196.00	22.00	246.00	270.00
132.00	163.00	64.00	55.00	156.00
122.00	254.00	140.00	76.00	130.00
124.00	200.00	20.00	124.00	55.00
228.00	225.00	0.00	0.00	0.00
Mean				
143.8 ± 20.24	208.4 ± 16.13	60.0 ± 24.18	164.40 ± 11.14	52.36 ± 16.14

Table 7: AST/ALT Ratios in The Test and Control Groups of Rats

Groups	Biochemical analysis		
	AST (U/L)	ALT (U/L)	AST / ALT Ratio
A	36.12 ± 10.13	143.11 ± 6.12	0.26
B	54.05 ± 12.12	196.12 ± 6.12	0.28
C	53.11 ± 11.10	59.13± 14.08	0.96
D	91.11 ± 13.14	164.11 ± 17.11	0.57
E	88.11 ± 16.19	152.15 ± 10.22	0.62

DISCUSSION

The qualitative studies of the above–mentioned extracts show the presence of many bioactive compounds, such as tannins which play a role in antitumor, antimicrobial, and antiviral activity [51, 52,53]. They have also been reported as an inhibitory agent in HIV replication [54]. They are important for the healing of mucous membranes [55]. The presence of cardiac glycosides indicated their use in many heart diseases [56]. The presence of phenols was indicated by many plant extracts. They act as antioxidants in humans as well as plants [57]. Saponins act as an important tool in the defence mechanism of plants as they inhibit microbial attacks, and they can be used for the treatment of fungal as well as yeast infections [58]. Alkaloids play an important role in many pharmacological actions. They can act as antimicrobial, antimalarial, anti-hyperglycaemic, and anti-cancerous agents [59]. They can be exploited for recreational drugs [60]. Terpenes could act as a therapeutic as well as a protective agent. They could be used in the agriculture industry for storing agricultural products [61]. Steroids play their role in the proper regulation of immune responses [62].

Phlobatannins show many astringent properties [63]. Carbohydrates provide strength for the different functions of the body; thus, we can say that they act as petrol to strengthen the body [64]. Due to the antimicrobial and anti-inflammatory actions of coumarins, they are thought to have anti-hyperproliferative effects in skin diseases [65]. Proteins' nutritional value could not be declined, as they act as central units or building blocks for life. For the maintenance of life and for a healthier body, proteins are a much-needed component [66].

Thus, the mean values for serum ALT in group A (no ART, no extract) was 36.12 ± 10.13U/L which is within the reference range of 18-45 U/L [67]. Also, the mean value for

serum AST in group A (no ART, no extract) was 143.11 ± 6.12U/L which is also within the reference range of 74-143 U/L.

However, reference values for ALT and AST in rats vary widely from laboratory to laboratory and from region to region [68]. ALT is more specific for the liver, but AST is often used to monitor the response of the liver to drug and other toxic substances especially in rats [69].

The serum ALT and AST values in groups A, B, D, and E are slightly raised, but none is raised up to five times (5x) above normal value. Therefore, the animals sustained mild disturbances of liver function and not toxic liver damage [70]. The AST/ALT ratio is useful for categorizing liver damage. Ratios greater than 1 are suggestive of liver damage [71, 72]. In contrast, Ajibade et al. [73] orally administered high doses of the aqueous extract of this plant on adult Wistar rats and recorded abnormal liver enzymes values.

In this study, the AST/ALT ratios are less than 1, suggesting minimal disturbances of liver function. There was no statistically significant difference between the ALT values for the test and control groups of rats as shown in table. 1 (p value > 0.999), but there was a statistically significant reduction (P value = 0.0160) table 2, in AST values in group C (extract alone) compared to those in group B (ART alone). This finding which demonstrated the hepatoprotective of the extract is in line with some earlier studies [74]. However, the addition of the extract to the ART in groups D and E did not confer hepatoprotection on these groups as shown in table. 4.

A possible explanation for this observation could be that the dose of extract added was suboptimal or that the duration of treatment was short. Usually, administration of hepatotoxic drugs to rats produces abnormal aminotransferases levels which peak within one week after initiation of treatment. This peak drops at four weeks and a steady level is maintained as long as ART treatment is continued [75].

Therefore, it seems that more time is needed for the hepatotoxicity to be reversed and the increased plasma aminotransferases to be restored to normal by the addition of the hepatoprotective extract. On the other hand, in some studies that produced hepatoprotection, the rats were pre-treated with the extracts before the hepatotoxic drugs were administered [76]. This pre-treatment with the extract probably stabilizes the hepatocytes before the administration of the hepatotoxic drugs since the mechanism of

hepatoprotection by these herbs is known to be through the production of antioxidants and heat shock proteins which stabilize cell membranes [77,78].

Pre-treatment with the extract before the initiation of ART should be tried in subsequent studies. The findings in this study may not actually simulate what will happen when HIV/AIDS patients are used as subjects in this study.

HIV/AIDS patients who usually have reduced plasma glutathione levels [79] are more likely to benefit from hepatoprotective herbs when on antiretroviral therapy. This is because such herbs are known to increase plasma glutathione level as already stated above. In addition to stabilizing the hepatocyte membrane, glutathione also boosts the immune system which is very important in hepatoprotection. It is obvious that the liver in such HIVAIDS patients will be more susceptible to hepatotoxic effects of antiretroviral drugs and will, therefore, benefit more when hepatoprotective herbs are used as therapeutic adjuncts in these patients.

The findings in the liver in this study are not surprising since the serum aminotransferases were not abnormally raised. This is in keeping with some findings that biochemical changes often occur earlier than histological changes in cases of liver disease [80].

CONCLUSION

This extract produced statistically significant reduction of serum aminotransferases in the group of rats that received extract alone. However, this reduction in serum aminotransferases was not observed in the groups that received antiretroviral drugs plus the extract. These statistical findings show that there was no statistically significant difference in serum ALT and AST between the exposed and control groups ($p > 0.05$). Consequently, it could be concluded that at the doses of ART and extract and length of exposure used in this study, aqueous leaf extract of Achyranthes aspera did not reduce the serum level of ALT and AST in rats. The reason for this could be suboptimal dose of extract or short duration of treatment. Perhaps, better results could be achieved if HIV/AIDS models of rats or HIV/AIDS patients are used as subjects in the study.

Competing Interests

Authors have declared that no competing interests exist

REFERENCES

1. Tobyn, G., Denham, A., & Whitelegg, M. (2010). The Western Herbal Tradition E-Book: The Western Herbal Tradition E-Book. Elsevier Health Sciences.
2. Ahmad, I., Ahmad Khan, M. S., & Cameotra, S. S. (2014). Quality assessment of herbal drugs and medicinal plant products. Encyclopedia of Analytical Chemistry, 46, 1-17.
3. Srivastav, S., Singh, P., Mishra, G., Jha, K. K., & Khosa, R. L. (2011). Achyranthes aspera-An important medicinal plant: A review. J Nat Prod Plant Resour, 1(1), 1-14.
4. Vijayan Arun, V.B. Liju, John J.V. Reena, B. Parthipan, C. Renuka. Indian Journal of Traditional Knowledge, 2007, 6(4), 589-594.
5. Tijani, A. Y., Uguru, M. O., & Salawu, O. A. (2008). Anti-pyretic, anti-inflammatory and anti-diarrhoeal properties of Faidherbia albida in rats. African Journal of Biotechnology, 7(6).
6. Pande, P. C., Tiwari, L., & Pande, H. C. (2007). Ethnoveterinary plants of Uttaranchal—A review.
7. Jitendra B. Jain, Sheetal C. Kumane, S Bhattacharya. Indian Journal of Traditional Knowledge. 2006, 5(2), 237-242.
8. The Wealth of India - Raw Materials, Council of Scientific & Industrial Research, New Delhi, 2005, 55-57.
9. Srivastava, D., & Shukla, K. (2016). Viral diseases on medicinal plants of north-eastern uttar pradesh. Plant Viruses: Evolution and Management, 89-129.
10. Paul, D., De, D., Ali, K. M., Chatterjee, K., Nandi, D. K., & Ghosh, D. (2010). Comparative study on the spermicidal activity of organic solvent fractions from hydroethanolic extracts of Achyranthes aspera and Stephania hernandifolia in human and rat sperm. Contraception, 81(4), 355-361.
11. Sutar, N. G., Sutar, U. N., Sharma, Y. P., Shaikh, I. K., & Kshirsagar, S. S. (2008). Phytochemical investigation and pharmacological screening of leaves of Achyranthus aspera Linn. as analgesic and antipyretic.
12. Singh, K., & Devi, P. U. (2013). In-vitro anthelmintic activity of methanolic and aqueous extracts of Achyranthes aspera Linn. (Amaranthaceae) stems. Int. J. Pharm. Sci, 3, 181-184.
13. Badgujar, S. B., & Mahajan, R. T. (2011). Indian Journal of Pharmaceutical Science & Research. Indian Journal of Pharmaceutical Science & Research, 1(1), 4-9.
14. Srivastav, S., Singh, P., Mishra, G., Jha, K. K., & Khosa, R. L. (2011). Achyranthes aspera-An important medicinal plant: A review. J Nat Prod Plant Resour, 1(1), 1-14.
15. Singh, V. K., Ali, Z. A., Zaidi, S. T. H., & Siddiqui, M. K. (1996). Ethnomedicinal uses of plants from Gonda district forests of Uttar Pradesh, India.
16. Gupta, P., Naudiyal, N., Raypa, P., & Kumar, V. A. (2024). Pharmacognostic Profile of a Potent Medicinal Herb: Nardostachys Jatamansi. In The Chemistry inside Spices & Herbs: Research and Development: Volume 4 (pp. 117-146). Bentham Science Publishers.
17. Jain, A., & Jain, S. K. (2016). Indian Ethnobotany: Bibliography of 21st Century (2001-2015). Scientific Publishers.
18. Anonymous. The Wealth of India - Raw Materials, Council of Scientific & Industrial Research (CSIR), New Delhi, 2007, 17-18.
19. W. Shibeshi, E. Makonnen L. Zerihun, A. Debella. African Health Science, 2006, 6(2), 108-112.
20. Mumoli N, Celi M, Cosimi A. Drug-related hepatotoxicity. N Eng J Med. 2006; 354(20):2191-2193.

21. Nunez M. Hepatotoxicity of antiretrovirals: Incidence, mechanisms, and management. Journal of Hepatology. 2006;44(1): S132-S139.
22. Nunez MJ, Martin-Carbonero L, Moreamaniego J, Gonzalez-castillo J. Impact of antiretroviral treatment-related toxicities on hospital admission in HIV infected patients. AIDS Res Hum Retroviruses. 2006; 22:825-829.
23. Bruno R, Sacchi P, Maiocchi L, Patruno S, Filice G. Hepatotoxicity and antiretroviral therapy with protease inhibitors: A review. Dig Liver Dis. 2006;38(6):363-373.
24. Heil EL, Townsend ML, Shipp K, Clarke A, Johnson MD. Incidence of severe hepatotoxicity related to antiretroviral therapy in HIV/HCV co-infected patients. AIDS Res Treat. 2010;856542, Epub.
25. Mulu W, Gidey B, Chemet A, Alem G, Abera B. Hepatotoxicity and associated risk factors in HIV-infected patients receiving antiretroviral therapy at Felege Hiwot Referral Hospital, Bahirdar. Ethiopia. Ethiop J Health Sci. 2013;23(3):217-226.
26. Gallant JE, Heffmann CJ. Hepatotoxicity. Johns Hopkins HIV Guide; 2013. Accessed 18 July, 2014.http://www.hopkinsguide.com/hopkins/ub/view/Johns_Hopkins_HIV_Guide/545091/all/hepatotoxicity
27. Becker S. Liver toxicity in epidemiological cohorts. Clin Infest Dis. 2004;38(S2): S49-S55.
28. Leist M, Gantner F, Kunstle G, Wandel A. Cytokine-mediated hepatic apoptosis. Rev Physiol Biochem Pharmacol. 1998;133: 109-155.
29. Nunez M. Clinical syndromes and consequences of antiretroviral-related hepatotoxicity. Hepatology. 2010; 52:1143-1155.
30. Anraku I, Rajasuriar R, Dobbin C, Brown R, Lewin SR, Suhrbier A. Circulating heat shock protein 60 levels are elevated in HIV patients and are reduced by antiretroviral therapy. PLoS ONE. 2012;7(9):e45291.
31. McRae MP, Lowe CM, Tian X, Bourdet DL, Ho RH, Leake BF, Kim RB, Brouwer KLR, Kashuba ADM. Ritonavir, saquinavir, and efavirenz, but not nevirapine inhibited bile acid transport in in human and rat hepatocytes. J Pharmacol Exp Ther. 2006; 318:1068-1075.
32. Kayode AAA, Kayode OT, Aroyeun OA, Stephen MC. Haematologic and hepatic enzyme alterations associated with acute administration of antiretroviral drugs. J Pharmacol Toxicol. 2011; 6:293-302.
33. Sule OJ, Godwin J, Nnopu IA. Biochemical investigation of hepatotoxic effects of antiretroviral drugs on Wistar albino rats. J Phys Pharm Adv. 2012;2(4):171-175.
34. Kalebic T, Kinter A, Poli G, Anderson ME, Meister A, Fauci AS. Suppression of human immunodeffiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and Acetylcysteine. Proc Natl Acad Sci USA. 1991;88(3):986-990.
35. Chen X, Mak IT. Magnessium supplementation protects against ritonavir mediated oxidative stress and hepatic eNOS downregulation. Free Radical Bio Med. 2014; 69:77-85.
36. Sleijfer S, Bannink M, Van Gool AR, Kruit WH, Stoter G. Side effects of interferon alpha therapy. Pharm World Sci. 2005;27(6):423-431.
37. Verma S, Thuluvath PJ. Complementary and alternative medicine in hepatology: Review of the evidence of efficacy. Clin Gastroenterol Hepatol. 2007;5(4):408-16.
38. Vergas-Mendoza N, Madrigal-Santillan E, Morales-Gonzalez A, Esquivel-Soto J, Esquivel-Chirino C. Hepatoprotective effect of silymarin. World J Hepatol. 2014;6(3):144-149.

39. Chen X, Mak IT. Magnessium supplementation protects against ritonavirmediated oxidative stress and hepatic eNOS downregulation. Free Radical Bio Med. 2014; 69:77-85.
40. Sleijfer S, Bannink M, Van Gool AR, Kruit WH, Stoter G. Side effects of interferonalpha therapy. Pharm World Sci. 2005;27(6):423-431.
41. Verma S, Thuluvath PJ. Complementary and alternative medicine in hepatology: Review of the evidence of efficacy. Clin Gastroenterol Hepatol. 2007;5(4):408-16.
42. Vergas-Mendoza N, Madrigal-Santillan E, Morales-Gonzalez A, Esquivel-Soto J, Esquivel-Chirino C. Hepatoprotective effect of silymarin. World J Hepatol. 2014;6(3):144-149.
43. Uhegbu FO, Elekwa F, Akubugwo EI, Chinyere GC, Iweala EEJ. Analgesic and hepatoprotective activity of methanolic leaf extract of Ocimum gratissimum. Research Journal of Medicinal Plants. 2012;6(1):108-115.
44. Becker S. Liver toxicity in epidemiological cohorts. Clin Infest Dis. 2004;38(S2):S49-S55.
45. National Research Council, NRC, (US) committee on guidelines for the use of animals in neuroscience and behavioural research; 2003. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Washington (DC): National Academies Press (US); A Sample Size Determination. Accessed 21 August 2015. Available: http://www.ncbi.nlm.nih.gov/book s/NBK43321/.
46. Haslam E. Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action. J. Nat. Prod. 1996; 59:205–215. doi: 10.1021/np960040+. [DOI] [PubMed] [Google Scholar]
47. Sule OJ, Godwin J, Nnopu IA. Biochemical investigation of hepatotoxic effects of antiretroviral drugs on Wistar albino rats. J Phys Pharm Adv. 2012;2(4):171-175.
48. Kayode AAA, Kayode OT, Aroyeun OA, Stephen MC. Haematologic and hepatic enzyme alterations associated with acute administration of antiretroviral drugs. J Pharmacol Toxicol. 2011; 6:293-302.
49. Uhegbu FO, Elekwa F, Akubugwo EI, Chinyere GC, Iweala EEJ. Analgesic and hepatoprotective activity of methanolic leaf extract of Ocimum gratissimum. Research Journal of Medicinal Plants. 2012;6(1):108- 115.
50. Pirie NW. Leaf protein; A beneficiary of tribulation. Nature. 1975; 253:239-243.
51. Frankel S, Reitman S. Determination of liver enzymes activity. Am J Clin Pathol. 1957; 28:56-58.
52. Haslam E. Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action. J. Nat. Prod. 1996;59:205–215. doi: 10.1021/np960040+. [DOI] [PubMed] [Google Scholar]
53. Khanbabaee K., Van Ree T. Tannins: Classification and definition. Nat. Prod. Rep. 2001;18:641–649. doi: 10.1039/b101061l. [DOI] [PubMed] [Google Scholar]
54. Kashiwada Y., Huang L., Kilkuskie R.E., Bodner A.J., Lee K.-H. New hexahydroxydiphenyl derivatives as potent inhibitors of HIV replication in H9 lymphocytes. Bioorg. Med. Chem. Lett. 1992;2:235–238. doi: 10.1016/S0960-894X(01)81071-4. [DOI] [Google Scholar]
55. Del-Rio A.O.B.G., BObdululio G., Casfillo J., Marin F.G., Ortuno A. Uses and properties of citrus flavonoids. J. Agric. Food Chem. 1997;45:4505–4515. [Google Scholar]
56. Braunwald E., Bloodwell R.D., Goldberg L.I., Morrow A.G. Studies on digitalis. IV. observations in man on the effects of digitalis preparations on the contractility of the

non-failing heart and on total vascular resistance. J. Clin. Investig. 1961;40:52–59. doi: 10.1172/JCI104236. [DOI] [PMC free article] [PubMed] [Google Scholar]
57. Dillard C.J., German J.B. Phytochemicals: Nutraceuticals and human health. J. Sci. Food Agric. 2000;80:1744–1756. doi: 10.1002/1097-0010(20000915)80:12<1744::AID-JSFA725>3.0.CO;2-W. [DOI] [Google Scholar]
58. Ejikeme C., Ezeonu C.S., Eboatu A.N. Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to nigerdelta area of nigeria. Eur. Sci. J. 2014;10:247–270. [Google Scholar]
59. •Cushnie T.T., Cushnie B., Lamb A.J. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int. J. Antimicrob. Agents. 2014;44:377–386. doi: 10.1016/j.ijantimicag.2014.06.001. [DOI] [PubMed] [Google Scholar]
60. Shi Q.I.U., Hui S.U.N., Zhang A.H., Hong-Ying X.U., Guang-Li Y.A.N., Ying H.A.N., Xi-Jun W.A.N. Natural alkaloids: Basic aspects, biological roles, and future perspectives. Chin. J. Nat. Med. 2014;12:401–406. doi: 10.1016/S1875-5364(14)60063-7. [DOI] [PubMed] [Google Scholar]
61. Rabi T., Bishayee A. Terpenoids and breast cancer chemoprevention. Breast Cancer Res. Treat. 2008;115:223–239. doi: 10.1007/s10549-008-0118-y. [DOI] [PubMed] [Google Scholar]
62. Sultana N., Ata A. Oleanolic acid and related derivatives as medicinally important compounds. J. Enzym. Inhib. Med. Chem. 2008;23:739–756. doi: 10.1080/14756360701633187. [DOI] [PubMed] [Google Scholar]
63. Shah B.A., Qazi G.N., Taneja S.C. Boswellic acids: A group of medicinally important compounds. Nat. Prod. Rep. 2008; 26:72–89. doi: 10.1039/B809437N. [DOI] [PubMed] [Google Scholar]
64. Setchell K.D.R., Cassidy A. Dietary Isoflavones: Biological Effects and Relevance to Human Health. J. Nutr. 1999; 129:758S–767S. doi: 10.1093/jn/129.3.758S. [DOI] [PubMed] [Google Scholar]
65. Theis N., Lerdau M. The Evolution of Function in Plant Secondary Metabolites. Bot. Gaz. 2003;164: S93–S102. doi: 10.1086/374190. [DOI] [Google Scholar]
66. Ojala T., Remes S., Haansuu P., Vuorela H., Hiltunen R., Haahtela K., Vuorela P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J. Ethnopharmacol. 2000; 73:299–305. doi: 10.1016/S0378-8741(00)00279-8. [DOI] [PubMed] [Google Scholar]