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Potential of Aqueous Extract of Jatropha Gossypiifolia Seed Against Highly Active Antiretroviral Therapy Induced Hepatotoxicity in Rats

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Abstract

While many methods exist for measuring the impact of AIDS, treatment remedy the human development approach focuses on people rather than medical or economic indicators. Human Development Index captures three basic dimensions of human development: a long and healthy life—measured by life expectancy at birth; knowledge—measured by adult literacy and school enrolment; and standard of living—measured by per capita gross domestic product. Thus, investigate the hepatoprotective activity of aqueous extract of Jatropha gossypiifolia seed in highly active antiretroviral therapy administered rats. Liver is a metabolically active organ responsible for many vital life functions. It performs many activities that are critical for survival. Due to its important activities, the liver is exposed to a number of insults and is one of

the body's organs most subject to injury. In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell. It is, therefore, necessary to search for effective and safe herbal drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety. Hence, the need to evaluate the aqueous extract of Jatropha gossypiifolia seed in highly active antiretroviral therapy (Lamivudine, Zidovudine and Efavirenz) administered rats. Sixty rats weighed between 150-200g were randomly divided into six groups and each group comprised of ten rats. Rats in group I were administered with distilled water. Rats in group II were administered with highly active antiretroviral therapy only. Rats in groups III - VI were administered 100, 200, 400 and 600 mg/kg Jatropha gossypiifolia seed plus highly active antiretroviral therapy respectively. The treatments were given orally for 28 consecutive days. On the 29th day, all rats were sacrificed under light diethyl ether anaesthesia; blood samples were collected for the assessment of biochemical parameters, while Serum levels of liver enzymes ALT, AST, ALP, and GGT were significantly ($p<0.05$) increased and albumin concentration was significantly decreased in animals treated with highly active antiretroviral therapy as compared to the normal control. Treatment of highly active antiretroviral therapy exposed animals with Jatropha gossypiifolia seed showed marked improvement in biochemical findings. Rise in liver enzymes was almost restored to normal in animals treated with Jatropha gossypiifolia seed. Jatropha gossypiifolia seed through its antioxidant activity effectively protects highly active antiretroviral therapy induced liver toxicity.

Keywords: Potential, Aqueous, Leaves, Extract, Jatropha Gossypiifolia, Seed, Antiretroviral, Therapy, Induced, Hepatotoxicity, Rats

INTRODUCTION

In the approximately 25 years since AIDS emerged as a major health emergency, the epidemic has had a serious, and in many places devastating, effect on human development. In some countries, AIDS is undermining progress towards the Millennium Development Goals, particularly those related to poverty reduction, achieving universal primary education, promoting gender equality, reducing child mortality and improving the health of mothers [1].

The scale of the epidemic's impact, highly varied from place to place, has been documented with increasing precision over the years as surveillance and analytical tools have improved. As a result, the interrelationship of AIDS with other problems of human development has

become clearer. The late Jonathan Mann's insight from the early 1990s—that AIDS shines a spotlight on human rights and societal issues—has been borne out in many ways, particularly in the epidemic's interactions with poverty, gender inequality and social exclusion [2].

Research over the past few years has shown how it exacerbates other major challenges to development, from the deterioration of public services and governance to humanitarian emergencies such as food insecurity and conflict. As a recent study of the relationship between AIDS and famine in southern Africa states, "HIV/AIDS accentuates existing difficulties, compelling us to confront many simultaneous problems, all of which need resolution" [3].

The impact of AIDS is still not fully understood, particularly when the long term is considered. The epidemic comes in successive waves, with the first wave being HIV infection, followed several years later by a wave of opportunistic diseases, and later still by a wave of AIDS illness and then death [4]. The final wave affects societies and economies at various levels, from the family and community to the national and international levels. None of the highly affected countries have yet hit the peak of the third wave nor advanced very far into the fourth, and as one study put it [5].

We don't know how severe the impacts of the third and fourth waves will be—little about this pandemic is linear and AIDS is a unique threat . . . What for example is the likely long-term damage—social, economic, psychological— wrought by the orphaning of millions of children? What we do know is that impacts will continue to be felt for years to come and the situation will get significantly worse before it gets better. Thus, the study 'Potentials of Aqueous Leaves Extract of Jatropha Gossypiifolia Against Highly Active Antiretroviral Therapy Induced Hepatotoxicity in Rats'

However, Liver is the largest organ in human body. It is a metabolically active organ responsible for many vital life functions. Liver plays a great role in carbohydrate, protein and fat metabolism, synthesis of bile components, detoxification of blood and storage of vitamins and minerals. It also performs many activities that are critical for survival such as synthesis of blood clotting factors, creation of proteins necessary for growth and metabolic processing of most drugs and toxins. It also has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the

biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [6].

To maintain a healthy liver is a crucial factor for overall health and well-being, however; it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [7].

Although antiretroviral drugs have significantly improved morbidity and mortality in HIV-infected patients, these benefits are compromised by numerous side effects, adverse clinical events and toxicities [8, 9]. Since the introduction of HAART, HIV infection has become a chronic disease. It has resulted in an increased prevalence and incidence of comorbidities among HIV-infected persons, requiring the use of more medications for longer periods. Therefore, it is not surprising that HAART associated toxicity, especially liver toxicity, has perhaps become one of the main limitations to treatment [8, 9].

Almost all antiretroviral drugs belonging to all available classes are responsible for an intrinsic liver toxicity, which is increased by the combined use of at least three different antivirals, in the so-called Highly active antiretroviral therapy (HAART) [10,11], despite the probability and extent of injury varies substantially with the individual agents [12].

In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cells. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for effective and safe drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety [7].

Many folk remedies from plant origin were tested for their potential antioxidant and hepatoprotective liver damages [6]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease. Aloe vera, Eclipta alba, Phyllanthus niruri, Jatropha gossypiifolia have been shown to possess anti-hepatotoxic properties.

Phytochemical screening of the seeds of Jatropha gossypiifolia have led to the discovery of many active principles. The general objective of this study is to investigate the hepatoprotective activity of aqueous extract of *Jatropha gossypiifolia* seeds in highly active antiretroviral therapy administered rats.

Figure 1: Jatropha gossypiifolia

MATERIAL AND METHODS

Plant Collection and Preparation of the Extracts Seed of Jatropha Gossypiifolia was obtained from Michika, Adamawa State. The taxonomic identity of the plant was verified at the Faculty of Agriculture, Federal University Wukari, Taraba State. Voucher specimen of the plant was kept at the University Herbarium.

The plant material was then carefully washed with distilled water to remove any extraneous materials, dried under shade at room temperature, grounded to a coarse powder using electronic grinder and the aqueous extract of the seeds of the plant was prepared by decoction as follows: 200g of the powdered seed of Jatropha Gossypiifolia was weighed by analytical balance. 1500mL of distilled water was added and boiled for 15 minutes with continuous stirring. After cooling, the solution was decanted and the supernatant solution was filtered with 0.1mm^2 mesh gauze. The filtrate was transferred into a Petri dish and was

frozen in a deep freezer overnight. On the next day the freeze extract was allowed to dry in a freeze dryer (lyophilizer) under vacuum pressure at lower temperature (-400C) and lower pressure (133x10-3mbar) for a week to obtain a freeze-dried product.

After the extract was dried, it was collected in air tight plastic containers, weighed, labeled and put in a desiccator for subsequent experiment [17, 18]. The weight of the dry extract was expressed as percentage of the total mass of dry plant powder to determine the percentage yield.

Phytochemical Screening Test

A preliminary qualitative phytochemical screening of the plant material was carried out employing the standard procedures [18,19,20,21,22] to reveal the presence of saponins, flavonoids, alkaloids, tannins, phenols, and glycosides. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a white coloured precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of yellow orange precipitate indicates the presence of alkaloids.

Detection of tannins; About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Detection of saponins; About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols; Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids; Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under

layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Preparation of Highly Active Anti-Retroviral Therapy

The three antiretroviral drugs used for the study (Lamivudine, Zidovudine and Efavirenz) were obtained from pharmacy store Modibo Adama University Teaching Hospital Yola. The drugs were combined at the doses of 26.46 mg/kg Lamivudine (3TC), 52.91 mg/kg Zidovudine (ZDV) and 52.91 mg/kg Efavirenz (EFV). The drugs were prepared by grinding the tablets into fine powder and dissolved in distilled water.

Extract preparation for the experiment

The graded concentrations of 100, 200, 400 and 600 mg/kg were prepared from Seed of Jatropha Gossypiifolia aqueous extract. HAART and extracts seed of Jatropha gossypiifolia were mixed together before administration. Only fresh drugs (prepared daily) were used.

Experimental Animals Preparation

The experimental animals used in this study were 60 Albino Wistar rats of both sexes, each weighing 150–200g and aged three months. All rats were maintained under the controlled conditions of temperature (25 ± 2◦C), humidity, and light (12 hours of light and dark) in the Animal House of Faculty of Basic Medical Sciences, Federal University Wukari. The animals had free access to food and clean tap water. The animals were housed in standard environmental conditions in stainless steel cages. The rats were acclimatised for 7 days before the start of the experiment. During the acclimatization the animals fed with Standard pelleted rat chow and water ad libitum. Ethical approval for this study was obtained from the Research Ethics Committee of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University Wukari.

Animal grouping and Drug dose

A modified method [26] was used for this test. In this study, 60 Albino rats were randomly allotted into one of the six experimental groups, and each group consisted of ten rats:

• Group I received only distilled water and served as a normal control (2mL).

• Group II received only HAART and served as a positive control (2mL.)

• Group III received combination of HAART and (100 mg/kg) extracts seed of Jatropha gossypiifolia

• Group IV received combination of HAART and (200 mg/kg) extracts seed of Jatropha gossypiifolia • Group V received combination of HAART and (400 mg/kg) extracts seed of Jatropha gossypiifolia • Group VI received combination of HAART and (600 mg/kg) extracts seed of Jatropha gossypiifolia.

Animals were deprived of food before drug administration after which they were allowed access to food. A volume of 2mL of each treatment was administered for each rat by oral intubation (blunt intragastric catheter or gavage) once a day in the morning at 9.00 a.m. for 28 consecutive days. The blunt intragastric catheter was cleaned, placed in an oven and sterilized after each administration to avoid any contamination. Toxicity signs and mortality were monitored daily.

Blood Sample Collection

At the end of the experiment, animals were fasted overnight and anesthetized with diethyl ether. Immediately each animal was placed in supine position on operating board. The extremities of the animals were stretched and fixed on a dissecting board. The abdominal cavity was opened and blood sample was withdrawn by cardiac puncture using sterile needle of 5ml syringe [23].

Assessment of Hepatoprotective Activity: In the present study the hepatoprotective activity was evaluated biochemically.

Biochemical Assay

Initially the blood samples were allowed to clot. Then the clotted blood was centrifuged (using Humax 4k bench top centrifuge) at 5000 revolution per minute (rpm) for 10 minutes to separate the serum. Serum marker enzymes of liver function: alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, alkaline phosphatase (ALP) activity, total bilirubin and total albumin were assayed using Automated Clinical Chemistry Analyzer (Cobas Integra 400plus).

Statistical Analysis

The data (expressed as mean ± SEM) were analysed by one-way ANOVA followed by Tukey–Kramer post hoc test using SPSS software version16.0 program. P values less than 0.05 were considered to be statistically significant.

RESULTS

Table 1: Phytochemical Screening of Crude Extract of Seed of Jatropha Gossypiifolia

S/N	TEST	COLOR OBSERVED	RESULT
1	Alkaloids (Dragendroff's)	Yellow orange ppt*	Positive
2	Alkaloids (Mayer's reagent)	White ppt*	Positive
3	Cardiac glycosides	Reddish brown	Positive
4	Anthranides	Yellow	Negative
5	Polyphenols	Green blue	Positive
6	Tannins	Blue black	Positive
7	Chromophores	Brown	Negative
8	Flavonoids	Yellow ppt*	Positive
9	Carotenoids	White	Negative
10	Saponins	Honey comb forth (foam)	Positive

Positive: secondary metabolite present; Negative: secondary metabolite absent; *ppt: precipitate

Percentage Yield from plant material Percentage yield (%Yield) of the crude extract of seed of Jatropha gossypiifolia was calculated by the following formula

$$\%\, Yield\ = \frac{weight\ of\ the\ aqueous\ extract\ obtained x 100}{Weight\ of\ the\ powder\ used\ for\ extraction}$$

$$\%\, Yield\ = \frac{29g x 100}{200g}$$

$$\%\, Yield\ = 14.5\%\ 9W/W)$$

Table 2: Comparison of the Mean ± SEM of the Biochemical Parameters

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Normal control	119.00±15.72a	85.77±13.45a	286.21±28.63a	4.23±0.43a	4.64±0.12a	0.03±0.12
Positive control	266.75±6.54	168.55±13.89	472.53±22.43	2.34±2.54	3.33±0.14	0.25±0.14
100mg/kg J. gossypiifolia + HAART	166.00±8.56*	99.37±9.32*	311.64±35.32*	5.89±0.32*	4.57±0.24*	0.09±0.11
200mg/kg J.	163.67±24.16*	97.13±9.31*	298.26±37.27*	5.87±0.65*	4.53±0.13*	0.06±0.23

gossypiifolia + HAART						
400mg/kg J.gossypiifolia +HAART	148.60±17.62*	93.64±2.87*	262.34±12.13*	5.24±0.67*	4.36±0.16*	0.04±0.27
600mg/kg J. gossypiifolia +HAART	145.00±13.43*	89.69±14.35*	226.55±8.49*	5.27±0.84*	4.45±0.12*	0.08±0.34

Phytochemical screening Test

The result of phytochemical screening of powdered crude extract of seed of Jatropha gossypiifolia showed the presence of many secondary metabolites (Table 1). The result revealed the presence of saponins, flavonoids, alkaloids, tannins, phenols and glycosides.

Effects of Jatropha gossypiifolia seed extract on the Biochemical parameters. The mean values of liver biomarkers (ALP, ALT, AST and GGT) increased significantly ($P < 0.05$) in HAART administered rats (group II) compared to rats in normal control group (Table 2).

On the other hand, mean values of albumin concentration decreased significantly ($P < 0.05$) in rats treated with HAART when compared to the rats in normal control group (Table 2). Administration of Jatropha gossypiifolia plus HAART (groups III – VI) significantly decreased ($P < 0.05$) the mean values of ALP, ALT, AST and GGT and significantly increased ($P < 0.05$) the mean value of albumin concentration when compared to positive control group (group II). Mean values of total Bilirubin has shown non-significant increase in HAART administered rats and decreased in Jatropha gossypiifolia extract plus HAART administered rats (Table 2).

Though there were no statistically significant differences among different doses of Jatropha gossypiifolia, slight decrease in mean values of ALT, ALP, AST, and GGT was observed as the concentration increases from 100 to 600 mg/kg.

DISCUSSION

Because of its functional roles in the body, liver is the major target organ of toxicity. Injury to the liver may affect the integrity of hepatocytes leading to the release of liver enzymes such as ALP, ALT, AST, and GGT since these enzymes are confined to hepatocytes and released into the blood following liver injury. Hence, these enzymes are commonly used as markers of hepatic injuries [24, 25].

In the current study, damage of the liver caused by HAART was evident by the alteration in serum marker enzymes and albumin concentrations. Administration of HAART (Group II) significantly increased the serum levels of liver enzymes (AST, ALT, ALP, and GGT) and significantly decreased albumin concentration. This result indicates liver cell damage; leakage of enzymes from cells and loss of functional integrity of cell. This is in consistent with the previous work [26] that indicated oral administration of rats with antiretroviral drugs (Efavirenz, Abacavir and Lamivudine) caused significant liver damage.

AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is a metabolic enzyme expressed primarily in the liver. Increase in serum ALT activity is typically associated with hepatocellular membrane damage and leakage of enzyme from hepatocytes [25]. Damage to the liver causes the release of ALT enzyme into the blood. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury. The increase in serum ALP activity is also associated with a pathological damage occurrence in the liver [27, 28].

When damage to heart or liver cells occurs, intracellular enzymes, such as AST, are released into the peripheral blood. Since AST is located in the parenchymal hepatic cells and heart muscle, this enzyme is used to assess damage to these areas.

Increases in AST can be seen in hepatitis, liver necrosis, cirrhosis, and liver metastasis [29]. Gamma Glutamyl Transferase (GGT) is also widely used to assess liver function. Some drugs, and also alcohol, induce the liver to produce more of this enzyme [30].

Total bilirubin results are comprised of the conjugated and unconjugated forms of bilirubin. Hyperbilirubinemia can occur in three areas as bilirubin is addressed by the body. In the prehepatic phase, increased bilirubin levels are caused by an increase in heme degradation and haemolysis. In the hepatic phase, increase levels are due to defective transport to the liver or defective conjugation by the liver. In the post hepatic phase, increase levels are due to defects transporting the conjugated bilirubin and bile out of the liver.

Therefore, total bilirubin measurements are used to diagnose and treat liver, haemolytic, haematological, and gallbladder obstructive disorders [31].

Albumin is a small protein which accounts for nearly 50% of the total plasma protein. Albumin is primarily synthesized by the hepatic parenchymal cells. Albumin is primary

function is the maintenance of colloidal osmotic pressure in the vascular and extravascular areas of the body and preventing edema. Additionally, albumin is a carrier transport protein. Hypoalbuminemia occurs in GI malabsorption, glomerulonephritis, nephritic syndrome, cirrhosis, severe burns, neoplasms, and autoimmune diseases. Low albumin levels indicate poor liver function and contribute to peripheral oedema and ascites sometimes seen in very late stage liver disease. Albumin levels are usually normal in chronic liver disease until significant liver damage is present [33].

According to this study a significant increase in the activities of serum enzymes with in eighteen hours of exposure of the rats to single dose of D-Galactosamine Lipopolysaccharide (D-GalN/LPS) induced hepatotoxicity in rats, indicating the severity of hepatocellular injury. Rats given DGalN/LPS elicited severe hepatic injury. Many folk remedies from plant origin were tested for their potential antioxidant and hepatoprotective liver damages [34]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease [35].

In this study, treatment with aqueous extract of Jatropha gossypiifolia seeds significantly lowered the values of liver enzymes elevated by HAART and restored the damaged hepatocellular architecture. The decreased level of liver biomarkers and restoration of hepatocytes in N. sativa treated group leads to the inference that Jatropha gossypiifolia seed aqueous extract counteracts the abnormal increase in serum enzymes and repair the hepatic tissue damage induced by HAART. These findings are in accordance with the finding of the previous study [36], where the aqueous extract of Jatropha gossypiifolia seed reported as an effective protector against carbon tetra chloride induced liver damage which was evidenced by decreasing elevated levels of liver enzymes and restoration of hepatocellular architecture. Similar works were also reported by other researchers [17, 37, 38].

The mean values of ALT, ALP, AST and GGT decreases as the concentration of N. sativa extract increases (Figures 7-10 respectively) which was supported by histopathological investigations (Figures 1-4 respectively) provided that hepatoprotective activity of aqueous extract of Jatropha gossypiifolia seed was enhanced with concentration and best activity was achieved at the dose of 600mg/kg.

It is chiefly the plant-based preparations which are employed for the treatment of liver disorders. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties [35].

Flavonoids have been reported to exhibit antioxidant, anti-inflammatory, and hepatoprotective activities. Furthermore, condensed tannins have been suggested to possess free radical scavenging, antioxidant, anti-inflammatory, and hepatoprotective activities, while saponins have been reported also to exhibit hepatoprotective activity via modulation of its antioxidant and anti-inflammatory activities.

Hepatoprotective activity of medicinal plants may also be due to synergistic action of flavonoids, condensed tannins, and saponins [37]. Therefore; the hepatoprotective activity exhibited by Jatropha gossypiifolia extract might be due to the anti-oxidative nature of the plant. The preliminary phytochemical analysis of the extracts in this study has also confirmed the presence of components such as flavonoids, tannins, saponins and phenolic compounds, which have been known for its antioxidant and hepatoprotective activities.

CONCLUSION

This study demonstrated that aqueous extract of Jatropha gossypiifolia seed can protect against HAART induced acute hepatotoxicity through restoration of the antioxidative Défense system and down-regulation of the pro-inflammatory pathway. Possible mechanism of hepatoprotective activity of Jatropha gossypiifolia may be due to its free radical scavenging and antioxidant activity as the result of the presence of flavonoids, tannins and phenolic compounds in the extracts.

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