Journal of Applied Life Sciences International

25(5): 9-19, 2022; Article no.JALSI.90753
ISSN: 2394-1103

Phytochemical Composition and Hepatoprotective Potential of Ethanolic Root Extract of *Jatropha curcas* in Acetaminophen-Induced Toxicity in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2022/v25i530304

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/90753

Original Research Article

Received 15 July 2022
Accepted 11 August 2022
Published 19 August 2022

ABSTRACT

This study evaluated the phytochemical composition and hepatoprotective potential of ethanolic root extract of *Jatropha curcas* in acetaminophen-induced toxicity in wistar albino rats. The phytochemical screening and composition were determined using gas chromatography. Thirty albino rats weighing between 170 and 200 g were used, were separated into 5 groups. Group one was administered distilled water, 2 was administered 1000 mg/kg AC only, 3, 4 and 5 were administered 1000 mg/kg acetaminophen + 200 mg/kg extract, 1000 mg/kg acetaminophen + 400 mg/kg extract, 1000 mg/kg AC + 100 mg/kg Silymarin. Phytochemical composition of root of the plant showed saponin (55.7079 µg/g) lunamarine (34.3976 µg/g), kaempferol (32.7107 µg/g), rutin (20.7399 µg/g), sapogenin (11.2644 µg/g), phenol (4.1557 µg/g), anthocyanin (1.1946 µg/g), epicatechin (0.8303 µg/g) and catechin (0.1883 µg/g). The plasma ALP, AST, ALT and GGT activities of the negative control were 151.50±14.11 U/L, 48.00±7.19 U/L, 79.50 ± 2.14 U/L and 3.50± 0.45 U/L respectively. The plasma ALP, AST, ALT and GGT activities of group 3 were 78.50± 4.75 U/L, 23.00± 2.35 U/L, 49.00± 3.65 and 2.95 ± 0.17 U/L respectively, were significantly decreased when compared with the controls. The plasma total protein, albumin and total bilirubin levels of the negative control were 57.00± 0.86 g/l, 32.00 ± 0.86 g/l, 20.35 ± 0.83 µmol. The plasma total protein, albumin and total bilirubin levels of group 3 were 61.50± 2.14 g/l,

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33.00± 0.86 g/l 11.15 ± 0.98 µmol respectively and were significantly increased when compared the controls. The significant improvement observed on the liver markers is suggestive of the hepatoprotective properties of *Jatropha curcas.*

Keywords: Jatropha curcas; acetaminophen; toxicity; liver markers; histology; wistar albino rats; phytochemicals.

1. INTRODUCTION

The liver is the major organ in human that performs more than two-third of the functions of the body [1] and serve as a frequent organ of attack by chemical pollutants and chemotherapeutic agents [2]. Cure for liver dysfunction or disorder is still considered as a controversial issue in the global society and in the non-appearance of an effective reliable therapeutic agent for prevention and treatment of liver disorders, numerous scientists are now turning to the utilization of hepatoprotective substances from medicinal plants or herbal agent [3,4].

Medicinal plants are endowed with countless of phytochemical constituents which ameliorate the physiological balance of man and the apprehension of these healing tendencies have been passed down from one generation to another [5]. In Africa, knowledge from traditional system of medicine is adopted by trado-medical practitioners to treat a vast number of illnesses [6,7]. Phytochemicals are non-nutritive bioactive ingredients locked up in medicinal plants that play protective function against external stress and pathogenic attack [8]. Based on their biosynthetic origin, they can be divided into several categories namely; phenolics, alkaloids, steroids, terpenes, saponins, isoflavonoids, anthocyanins, anthraquinones, glycosides, etc and can elicit antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties [9].

Phytochemicals have been reported to elicit curative potential against neurological disorders, anti-inflammatory effect, protection against lipid peroxidation and free radicals generated by chemical agents [10]. Farnesol, farnesyl acetone, hexahydrofarnesyl acetone, pristine, and squalene have been to yield antioxidant properties against oxidative stress [11]. Based on their biosynthetic origin, they can be divided into several categories namely; phenolics, alkaloids, steroids, terpenes, saponins, isoflavonoids, anthocyanins, anthraquinones, glycosides, etc and can elicit antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties [9].

The therapeutic properties of medicinal plants lean back in the mineral elements such as (Cl)) or micro (manganese (Mn), zinc (Zn), iron (Fe), potassium (K), magnesium (Mg), copper (Cu), cobalt (Co) and chromium (Cr) [11,12,13], embodied in them which is responsible for their clearly defined physiological action in human [14] and this make it vital to investigate on the mineral profile of medicinal plants [15].

Jatropha curcas (physic nut), belonging to *Euphorbiaceae* family, is a medicinal plant with several curative properties against bacterial and fungal diseases [2]. *Jatropha curcas* is a diverse subtropical and tropical genus which includes succulents, caudiciform species, herbaceous perennials and woody tree [6]. The biofuel, water infiltration, livestock protection and phytoremediation of various contaminated soils of *Jatropha curcas* have been reported [16,17,18]. The diverse beneficial properties of shown in various publications and those claims made by practitioners of traditional system of medicine could be attributed to the chemical composition, hence the need to carry out the phytochemical screening, mineral compositions and the protective potential of the root of medicinal plants including *Jatropha curcas* becomes significant which is the basis of this research.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

All the reagents used in the study were of analytical grade. They were obtained from Sigma Fine Chemicals Limited, Upsalla, Sweden, Fluka Chemical Company Plc Germany. Reagent kits for enzymes assays were purchased from Randox Laboratories Ltd., United Kingdom. All solutions, buffers and reagents were prepared with glass-distilled water.

2.2 Quantitative Phytochemical Screening

Quantitative phytochemical screening of the coarse powdered plant's roots was carried out using BUCK M910 Gas Chromatography equipped with a flame ionization detector. A RESTEK 15 Meter MXT-1 column (15 µm x 250 µm x 0.15 µm) was used. The injector temperature was 280^{o}C with splitless injection of 2µl of sample and a linear velocity of 30cms^{-1}. Helium 5.0pa.s was the carrier gas with a flow rate of 40m/min. The oven operated initially at 200^{o}C, was heated to 330^{o}C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 15minutes, the detector operated at a temperature of 320^{o}C. Phytochemicals in the plant's roots were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in µg/g.

2.3 Antioxidant Minerals Analysis

Antioxidant mineral content of the root of the plant sample was determined using Atomic Absorption spectrophotometer method, following the procedure as described by the American Public Health Association (1995). Exactly, 50 g of the plant sample was weighed and transferred into a glass beaker of 250 ml volume, containing 10 ml of concentrated nitric acid was added and heated to boil till the volume is reduced to about 15-20 ml, by adding 5 ml of concentrated nitric until all the residues were completely dissolved. The mixture was cooled and made up to 100 ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

2.4 Collection of Plant Material

The entire plant with it roots were collected from the botanical garden of the Department of Pharmacognosy and Phytotherapy University of Port Harcourt. The plant sample was it was identified by Dr. Suleman at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/PSB/2017/026, while all forms of other plant roots were separated from the roots of the plant.

2.5 Drying, Grinding and Extract Preparation

The harvested plant roots were washed with water and subjected to shed-drying for eight weeks. The shade-dried roots were then grinded into coarse powdered form using a grinder from Choba motor factory, Port Harcourt. The coarse root powdered sample was stored in a sealed container and kept in a dark, cool and dry container. However, 250 g of the coarse root powdered sample was macerated in 500 mL of ethanol (C_2H_5OH). The container was covered and kept for fourteen days. The mixture was then subjected to occasional stirring and shaking. The mixture was then filtered by cotton. The resulting filtrate was finally filtered using a Whatman filter paper grade 1 (542 mm). The filtrate was condensed and evaporated to dryness using a rotary evaporator and water bath at 50˚C. The ethanolic root extract which weighed 85g was stored in air-tight containers in a refrigerator until when required for analysis.

2.6 Experimental Animals

Thirty (30) wistar albino rats of both sexes, weighing between 170 and 200 g were used for this study. The rats were purchase from the Department of Pharmacy, University of Port Harcourt Choba. They acclimatized for 14 days in the Biochemistry Animal House University of Port Harcourt and the rats were separated into five (5) groups, six rats per group which were treated as follows:

Group 1: Received rat + feed + + dist. H_2O only, serving as positive control.
Group 2: Received + 1000 mg/kg ACPH + dist. H_2O only, serving as negative control.
Group 3: Received +1000 mg/kg ACPH + dist. H_2O + 200 mg/kg Extr, serving as test 1.
Group 4: Received +1000 mg/kg ACPH + dist. H_2O + 400 mg/kg Extr, serving as test 2.
Group 5: Received +1000 mg/kg ACPH + dist. H_2O +100 mg/kg silymarin, serving reference treatment group.

Note: ACPH= acetaminophen

2.7 Collection of Blood Sample

After administration of the extract, acetaminophen and the reference drug (silymarin) for 30 days, on the 31st day, the rats

were humanly sacrificed by cervical dislocation. Blood sample were collected for biochemical assays while the liver was harvested and preserved with formaldehyde for histological examination.

2.8 Statistical Analysis

Data represented in means ± standard deviation (M±SD) were analyzed using Statistical Package foe Social Sciences (SPSS) for window version 16 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparison was done using Turkey Post hoc at ($p \leq 0.05$) confidence interval.

3. RESULTS AND DISCUSSION OF FINDINGS

3.1 Phytochemical Screening and Quantification

Wellington et al. (2019) in their report on chemical composition of phytochemical and essential oil of *Euphorbia heterophylla*, showed the various sub-members of alkaloids, saponins, anthocyanins, anthraquinones, glycosides, sterol, phenolic acids, terpenoids, lignans and essential oils present in Euphorbia heterophylla which they claim are responsible its therapeutic properties. In this study, the phytochemical screening and quantification of the root of *Jatropha curcas* revealed the presence of ten phytochemical constituents namely; anthocyanins, oxalate, rutin, phenol, epicatechin, linamarin, sapogenin, saponin, phytate, kaempferol and catechins. Saponin (55.71 µg/g) was highest in concentration followed by linamarin (34.40 µg/g), kemferol (32.71 µg/g), rutin (20.74 µg/g), sapogenin (11.26 µg/g), phenol (4.16 µg/g) while the least in concentration was Catechincatechin (0.18 µg/g) as shown in Table 1.

Table 1. Quantitative phytochemical of *Jatropha curcas* ethanolic root extract

Phytochemical	Concentration (µg/g)
Anthocyanin	1.1946
Oxalate	1.0557
Rutin	20.7399
Phenol	4.1557
Epicatechin	0.8303
Linamarin	34.3976
Saponin	55.7079
Sapogenin	11.2644
Phytate	0.6996
Kaempferol	32.7107
Catechin	0.1883

Saponin has been reported by many researchers to antifungal, insecticidal, cytotoxic, anti-inflamaotry, immunostimulant, hypercholesterolemic and hypoglycemic effects [17,18,19]. Several investigations have showed that saponins, especially those of ginseng and soy elicit cytotoxicity against different types of cancer lines including Hep-G2 (hepatocellular carcinoma cell line), HT1080 (fibrosarcoma cell line), HeLa (cervical cancer), HL-60 (promyelocytic leukemia cells) and MDA-MB-453 (breast cancer) as reported by [19] and [20]. In this study, high saponin concentration was observed in the root of *Jatropha curcas.* The high saponin content characterized in *Jatropha curcas* indicate that herbal drugs can be derived from the plant's roots with anti-infalmmatory, hypercholesterolemic and hypoglycemic potency.

Linamarin as a nitriloside containing vitamin B17 usually releases a powerful cytotoxin, the HCN upon hydrolysis, which can be detrimental to neoplastic cells (cancer cells) which is almost completely deficient of the detoxification enzyme (rhodenase) [21,22]. Linamarin which chemically known as 2-(-D-glucopyranosyloxy)-2-methylpropanitrile whose cyanide ions (CN-) upon hydrolysis by linamarase to produce glucose, acetonecyanohydrin, which later decomposes to hydrogen cyanide and acetone [23,24]. Iyuke et al*.* [23] when stating the structural similarity between methyl--glucoside and linamarin of showed linamarin can transport glucose into the cells of the body as well. In this study, the high linamarin content observed in the roots of *Jatropha curcas* is suggestive that the plant could be a novel source of anti-neoplastic agent and can aid insulin function in mobilizing glucose into the liver which agrees with the claims of Iyuke et al*.* [23]. Kemferol was next to linamarin in concentration as shown in Table 1. Kemferol is a flavonoid member which has been proven to possess antidiabetic and anticancer properties [25]. The anticancer effects of flavonoids (flavonols, flavanols, anthocyanins, flavanones and isoflavones) as been shown to be attributed to the capacity of these bioactive compounds to inhibit cell cycle, cell proliferation and oxidative stress, as well as to induce enzyme detoxification and apoptosis [26].

3.2 Antioxidant Mineral Profile

The antioxidants minerals found in *Jatropha curcas* roots were Iron (0.449 ppm), Zinc (2.323 ppm), Copper (0.375 ppm), Selenium (2.049 ppm) and Manganese (3.462 ppm). Manganese

was highest in concentration followed by selenium, zinc, iron while the least was copper as presented in Table 2.

Table 2. Antioxidant minerals of *Jatropha curcas* roots

Minerals	Concentration (ppm)
Iron	0.449
Zinc	2.323
Copper	0.375
Selenium	2.049
Manganese	3.462

Rehnberg et al. [27] stated that manganese is an important element and is a constituent of metalloenzymes that oxidize cholesterol and fatty acids. The European Food Safety and Authority [28] reported that a deficiency in manganese can cause bleeding disorders, while excessive amounts can cause speech disorders, leg cramps, and encephalitis. The manganese level in the root of *Jatropha curcas* is reflective that the plant can be used for managing bleeding disorder. According to the National Institute for Health [29], Copper that is found in various human transcription factors and enzymes and is considered as an important essential trace element. Plum et al. [30], showed that zinc is an essential element, and cellular zinc promotes homeostatic control to avoid accumulation of surplus zinc. Exposure to excessive zinc results in copper deficiency and cell apoptosis. In addition, zinc deficiency has been linked to a suppressed immune response as reported by Plum et al. [31]. Pathak and Kapil [32] reported that zinc is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions. Calcium is reported to be essential for blood clotting, bone and teeth formation and as a co-factor in some enzyme catalysis [33]. In humans, magnesium is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium [34]. The presence of manganese and copper in the roots of *Jatropha curcas* is reflective of Zinc and copper could be considered important for metabolizing glucose and lowering cholesterol.

3.3 Effects of Ethanolic Root Extract of *Jatropha curcas* on Liver Biomarkers

The effects of ethanolic root extract of *Jatropha curcas* on the plasma liver biomarkers are presented in Tables 3 and 4.

Table 3. Effect of ethanolic root extract of *Jatropha curcas* on AST, ALT, ALP and GGT of Acetaminophen induced Liver damage in albino rats

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Positive Control	91.50 ± 1.34^{a}	23.00±0.86^{a}	64.00± 4.10^{a}	2.45± 0.10^{a}
Negative control	151.50±14.11^{b}	48.00±7.19^{b}	79.50 ± 2.14^{b}	3.50± 0.45^{b}
AC1000 mg+200 mg/kgExt.	78.50± 4.75^{ab}	23.00±2.35^{ab}	49.00± 3.65 ^{ab}	2.95 ± 0.17 ^{ab}
AC1000 mg+400 mg/kgExt	67.50 ± 2.57 ^{ab}	22.67±1.52^{ab}	43.00± 0.86 ^{ab}	2.85± 0.13 ^{ab}
AC1000 mg+ SY100 mg/kg	88.50 ± 9.20 ^{ab}	25.00±1.53^{ab}	45.50± 5.19 ^{ab}	2.55 ± 0.17 ^{ab}

AC= Acetaminophen, Ext= Extract, SY= Silymarin

Data are reported as mean ± standard error (M ± SEM), n =6

Superscript bearing "^{b}" indicates a statistical significant difference ($p < 0.05$) when compared to the positive control and superscript bearing "^{ab}" shows significant difference at $p < 0.05$ when compared to positive and negative control values down the groups

Table 4. Effect of ethanolic root extract of *Jatropha curcas* on total protein, Albumin and total bilirubin of Acetaminophen induced Liver damage in albino rats

Group	Total Protein (g/l)	Albumin (g/l)	Total bilirubin (µmol)
Positive control	69.00± 0.86^{a}	37.50 ± 1.33^{b}	16.65 ± 1.17^{a}
Negative control	57.00± 0.86^{b}	32.00 ± 0.86 ^{b}	20.35 ± 0.83 ^{b}
AC1000 mg+200 mg/kgExt	61.50± 2.14 ^{ab}	33.00± 0.86 ^{ab}	11.15 ± 0.98 ^{ab}
AC1000 mg+400 mg/kgExt	64.00 ± 0.86 ^{ab}	33.00 ± 0.86 ^{ab}	12.80 ± 0.76 ^{ab}
AC1000 mg+ SY100 mg/kg	62.50 ± 0.76 ^{ab}	35.00 ± 0.86 ^{ab}	15.62 ± 0.40 ^{ab}

AC= Acetaminophen, Ext= Extract, SY= Silymarin

Data are reported as mean ± standard error (M ± SEM), n =6

Superscript bearing "^{b}" indicates a statistical significant difference ($p < 0.05$) when compared to the positive control and superscript bearing "^{ab}" shows significant difference at $p < 0.05$ when compared to positive and negative control values down the groups

Acetaminophen is an antipyretic and analgesic drug used widely in clinics. When used at therapeutic doses, APAP is metabolized by glucuronidation or sulfation by the cytochrome p450 system into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [32]. In situation of normalcy, NAPQI is rapidly converted to nontoxic metabolites by glutathione (GSH). However, at doses, NAPQI levels increase and may react with hepatic proteins, resulting in liver injury [33]. Presently, diagnosis of APAP overdose is based on raised APAP levels in peripheral blood [34], elevation of the activities of liver marker enzymes including alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and estimation of APAP ingested [32]. In this study, a significantly increased plasma AST, ALT, ALP and GGT activities in the negative control was observed following administration of 1000 mg/kg b.wt of acetaminophen when compare to the positive control values as shown in Table 4. The significantly increased plasma AST, ALT, ALP and GGT activities observed is suggestive of liver damage and which agrees with Kane et al. [35] on acetaminophen-induced liver toxicity in rats. However, a significantly decreased plasma AST, ALP, ALT and GGT was observed following administration of 1000 mg/kg bwt of acetaminophen with 200 and 400 mg/kg b.wt of the ethanol extract of *Jatropha curcas* when compared to the controls. The significantly decreased plasma AST, ALT, ALP and GGT activities observed in the extract and acetaminophen administration is suggestive of the capacity of the extract to protect against liver damage. These results agrees with the report of Maryam et al. [34] on an invivo study on the hepato-protective effects of Crocus sativus, Ziziphus jujuba and Berberis vulgaris against acute acetaminophen and rifampicin-induced hepatotoxicity.

Albumin, total bilirubin and total protein are mixtures of molecules that can be used to evaluate the normal function while decreased serum albumin and total protein concentration is a pointer of liver damage [36]. Bilirubin is an important metabolic product of blood with biological and diagnostic values and increased serum levels of bilirubin is reflective of liver toxicity [37]. In this study, the decreased plasma concentration of albumin and total protein and the increased serum levels of bilirubin observed in the negative control are indicative of liver damage facilitated by exposure to acetaminophen administration. However, the dose-dependent increases on the plasma level of group 3 and 4 administered 1000 mg/kg b.wt acetaminophen plus the extract at 200 mg/kg b.wt and 400 mg/kg when compared to the

Plate 1. Photomicrograph of Liver tissue of control (Distilled water only) group treated for 30 days (H&E X100), shows no histological change

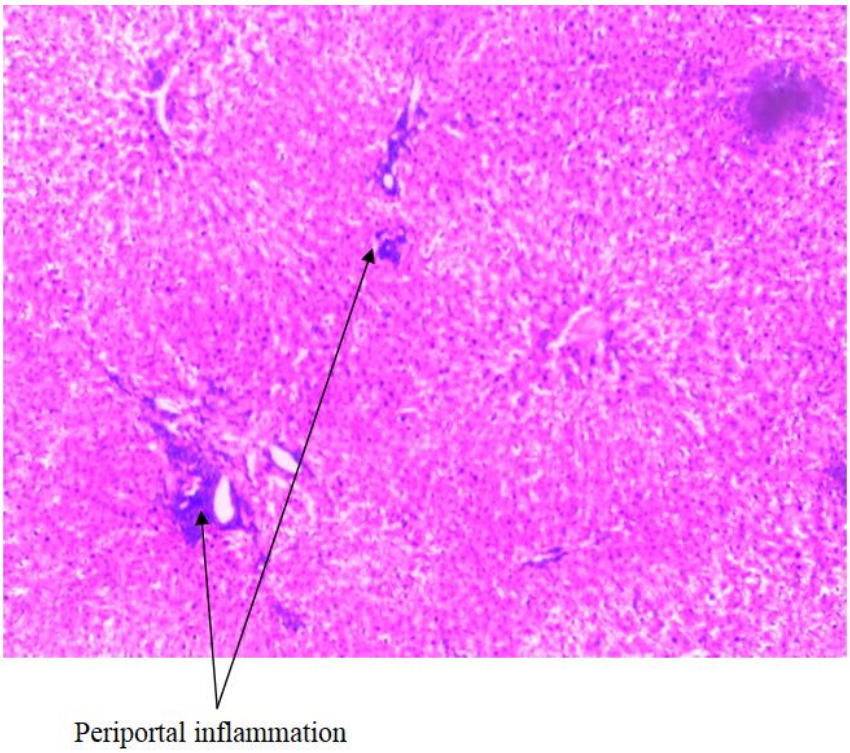

Plate 2. Photomicrograph of liver tissue of control (1000 mg/kg Acetaminophen) group treated for 30 days (H&E X200), shows periportal inflammation

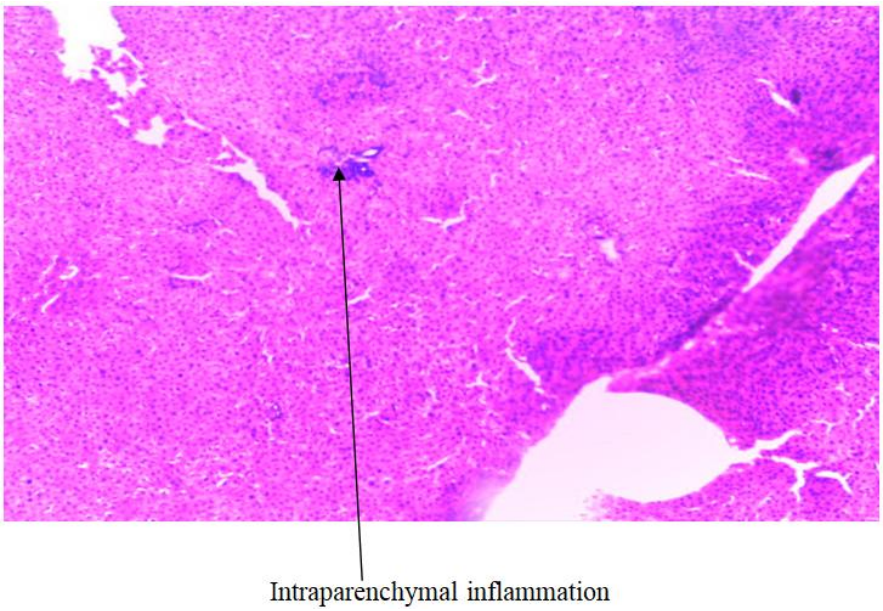

Plate 3. Photomicrograph of liver tissue of 1000 mg/kg Acetaminophen + 200 mg/kg *Jatropha curcas* root Extract treated group for 30 days (H&E X100), shows recovering intraparenchymal inflammation

negative and positive control values are indicative of the preventive effect of root ethanolic extract of *Jatropha curcas.* These results also support the work of Pendota et al. [39] on the effect of administration of aqueous extract of *Hippobromus Pauciflorus* leaves in male wistar rats.

Histological examination showed improved in the arcetecture of the liver rats administered with acetaminophen and 200 and 400 mg/kg b.wt of the extract when compared to the positive and negative control as shown Plates 1 - 5.

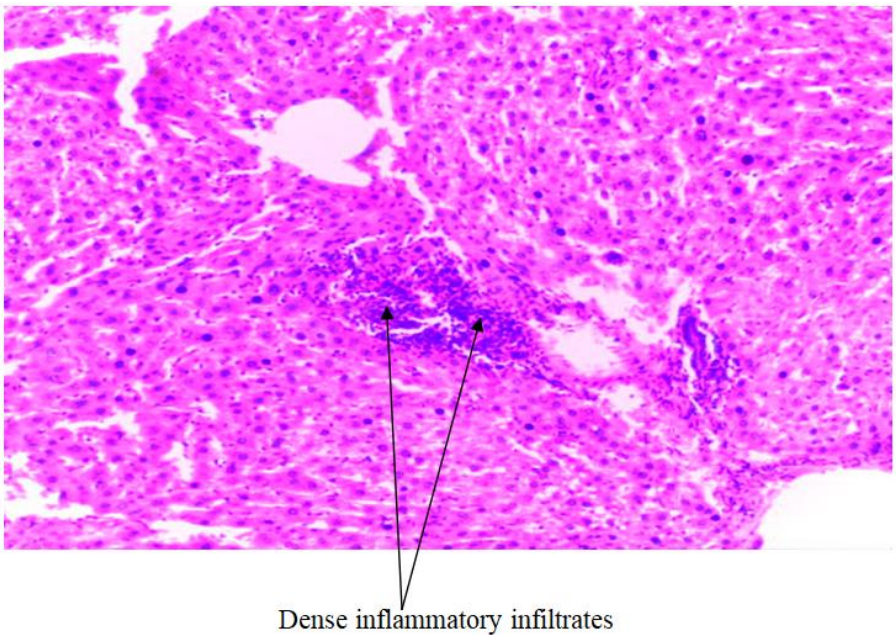

Plate 4. Photomicrograph of liver tissue of 1000 mg/kg Acetaminophen + 400 mg/kg *Jatropha curcas* root Extract treated group for 30 days (H&E X 300), shows recovering dense periportal inflammation

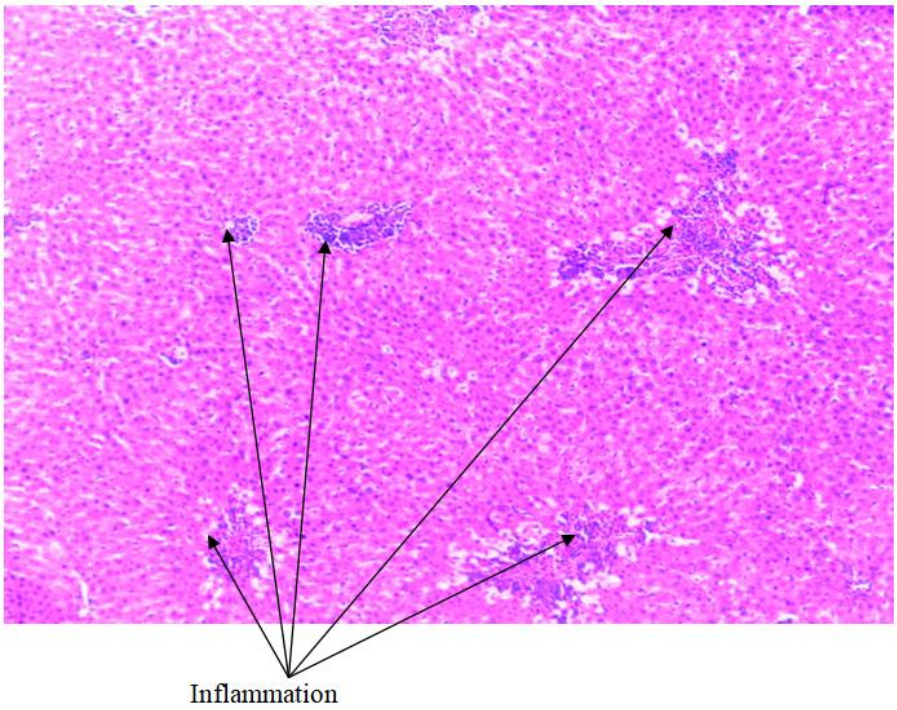

Plate 5. Photomicrograph of liver tissue of 1000 mg/kg Acetaminophen+100 mg/kg sylimarin treated group for 30 days (H&E X 200), shows multiple focus of intraparenchymal and portal inflammation

4. CONCLUSION

Medicinal plants yield several therapeutic and nutritional properties which attributed to the antioxidant phytochemical constituents and antioxidant mineral profile found in them. Screening for antioxidant minerals and phytochemicals of the roots of *Jatropha curcas* showed the presence of anthocyanins, oxalate, rutin, phenol, epicatechin, linamarin, sapogenin, saponin, phytate, kemferol and catechins phytochemicals and iron, zinc, copper, selenium and manganese. Administration of acetaminophen significantly increased the plasma bilirubin and liver enzyme activities as well decreased the plasma total protein and albumin concentration. The significant improvement observed on the plasma liver enzyme activities, total protein and albumin concentrations following administration of acetaminophen with two graded doses of ethanolic root extract of *Jatropha curcas* is indicative of its hepatoprotective potential.

ACKNOWLEDGEMENT

The author expressed his sincere gratitude to Mr. Wellington Emmanuel Ogbomade who ensure that this manuscript is properly written, Dr (MRS) Ogunka-Nnoka, C.U, who created time to supervised this study and the Department of Biochemistry University of Port Harcourt for their kind contribution towards the success of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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