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Ethanol Extract *of Acalypha wilkesiana Muel Arg* Leaves Ameliorates Paracetamol-induced Hepatotoxicity in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author CCM designed the study, wrote the protocol and supervised the work. Authors CLO and SIO carried out all laboratories work and performed the statistical analysis. Author CLO managed the analyses of the study. Author CLO wrote the first draft of the manuscript. Author CCM managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: The effect of ethanol leaf extract of *A. wilkesiana* on the liver of paracetamol- induced hepatotoxicity was investigated in wistar albino rats.
Study Design: Animal experimental study.
Place of Study: Department of Biochemistry, Faculty of Biological science University of Port Harcourt P.M.B 5323 Port Harcourt Nigeria
Methods: Liver toxicity was induced with 2000 mg/kg body weight of paracetamol (PARA) orally. The extract was administered to paracetamol treated wistar albino rats at a dose of 100 mg/kg, 200 mg/kg and 300 mg/kg body weight. Qualitative phytochemical analysis of the leaves of *A. wilkesiana* (AW) showed that they are rich in flavonoids, phenols, tannins, terpenoids, cardiac

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glycosides, protein, alkaloids and steroids. The effect of the ethanol leaf extract of *A. wilkesiana* on the liver was monitored by measuring the liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase), total bilirubin, albumin and total protein.
Results: The ethanol leaf extract of *A. wilkesiana* significantly (p<0.05) lowered the activities of alkaline phosphatase, alanine and aspartate transaminases.
Conclusion: Histopathological studies showed that ethanol leaf extract of *A. wilkesiana* had a therapeutic effect on the liver of wistar albino rats with paracetamol induced hepatotoxicity.

Keywords: Acalypha wilkesiana; hepatotoxicity; paracetamol.

1. INTRODUCTION

Acalypha wilkesiana also known as copperleaf is an ever green shrub and mainly planted in some parts of Nigeria as an outdoor plant. It is mainly used by the Yoruba speaking part of Western Nigeria for the treatment of skin infections most especially in new born. *A. wilkesiana* has been reported to have anti-cancer, anti-malarial [1], anti-diabetic, anti-fungal, anti-microbial, anti-inflammatory and anti-pyretic effect [2]. Phytochemicals are plant chemicals which are found in plants. Some of them serve as antioxidants while some of them contain toxins which have potential side effects on the body. Ricin which is found in castor bean is toxic and can kill if the castor bean seed is eaten raw. Antioxidants inhibit the oxidation of free radicals that may cause cellular damage and cancer. Phytochemicals such as carotenoids acts as scavanger for free radical that damage the body tissues, Lycopene, a carotene which gives red colour to fruits (tomatoes, water melon) and vegetables have been linked to a lower risk of prostate cancer [3]. Various researches have shown that phytochemicals including carotenoids and flavonoids which are mainly found in colourful fruits and vegetables have potentials to reduce risk of cancer, stroke and other diseases. Paracetamol is a potential analgesic which is popularly used for the treatment of headaches and body pains. When paracetamol is taken in excess toxicity results due to the accumulation of its metabolite N-acetyl-para-benzoquinone imine which is toxic to the liver [4,5,6,7]. This is due to depletion of the liver's glutathione which conjugates N-acetyl-para-benzoquinone imine to cysteine and mercapturic acid.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample and Preparation of Leave Extract

The leaves of *A. wilkesiana* plant used for this research were obtained from Abuja park of University of Port Harcourt.

After due identification at the Herbarium of the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria as *Acalypha wilkesiana muell Arg.* Leaves of *Acalypha wikesiana* were washed and shade dried, after which the leaf powder was prepared using home grinder/blender. One thousand grams (1000 g) of the powdered *A. wilkesiana* was weighed and soaked in 3000ml of 95% ethanol for 48 hours after which it was sieved using a muslin cloth and afterwards filtered with Whatmann paper size 1. The filtrate was concentrated using Rotary evaporator at 45ºC, the weight of the concentrates were taken and the percentage yield calculated and kept at 4ºC until usage.

2.2 Determination of Phytochemical Content

Twenty grams (20 g) of the homogenized sample was mixed with 60 g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was placed in a 500 ml beaker; 300 ml of n-hexane was added for extraction to be carried out using EPA 354ºC method for 48 h. Crude extract obtained was evaporated to dryness using a rotary vacuum evaporator at 40ºC. The residue was transferred with n-hexane into a 5 ml florisil column for clean-up.

2.3 Experimental Design for Hepato-therapeutic Study

2.3.1 Animals

A total of 56 both male and female wistar albino rats was weighing 150-200 g were used in the study. Research was carried out according to the rules and regulations guiding the use of animals. The animals were sorted into six groups with nine (9) animals each. The animals were grouped as in the Table 1.

Three rats in group 1 served as control (without paracetamol (PARA), untreated), group 2 served

as disease control (APAP + water). Rats in group 3, 4 and 5 received paracetamol and oral administration of ethanol leaf extract of *A. wilkesiana* (A.W) 100, 200 and 300 mg/kg body weight respectively for three weeks. Treatment started 48 hours after they received paracetamol and lasted for 21 days. Mode of administration was adopted from the work done by Ikewuchi [8]. Three (3) rats (n=3) were sacrificed from each group at seven (7) days interval during treatment for three (3) weeks. The animals were anaesthesized using chloroform. The animals while under anaesthsia were painlessly sacrificed and the blood sample were collected into Heparin bottles for Chemistry test and the liver was collected and preserved in 10% Formal-saline solution for Histological examination.

Table 1. Group description

Groups	Treatment
1	Control (without PARA, Untreated)
2	Disease control (PARA 2000 mg/ kg body weight)
3	PARA 2000 mg/kg body weight + 100 mg/kg body weight AW
4	PARA 2000 mg/kg body weight + 200 mg/kg body weight AW
5	PARA 2000 mg/kg body weight + 300 mg/kg body weight AW
6	PARA 2000 mg/kg body weight + 25 mg/kg body weight sylimarin

2.4 Determination of Plasma Hepato-specific Enzymes

The plasma activities of alkaline phosphatase, alanine and aspartate transaminases were determined using Mindray test kits. The activities of alanine and aspartate transaminases were monitored at a wavelength of 540 nm while that of alkaline phosphatase was monitored at a wavelength of 405 nm. The plasma total bilirubin concentration was determined using Mindray test kit and was measured at a wavelength of 578 nm.

2.5 Histopathological Study on the Liver

The histopathological study of the liver was carried out by Dr. Paul S.C. of the department of Anatomy, University of Port Harcourt Nigeria. The biggest lobe of the Liver was cut off with surgical blade and placed in a sample bottle containing 10% Formal-saline solution for Histological examination. Liver sections were prepared and stained with Haematoxylin and Eosin after which the stained section was examined under light microscope.

2.6 Ethical Approval

The protocol of this study was approved by the research ethical approval committee of Department of Biochemistry, University of Port Harcourt with authorization number: UPH/BCH/ REC/015/027.

2.7 Statistical Analysis

Means and SEMs were calculated for all data. Significant differences between means were evaluated using Post Hoc Turkey. A difference was considered significant when *p* was less than 0.05. Data analysis was carried out using Microsoft Excel (2010) Microsoft Corporation, Seattle, WA, (USA) and Statistical Package for Social science (SPSS) version 16 Inc., Chicago, IL USA.

3. RESULTS AND DISCUSSION

The preliminary qualitative screening revealed the presence of saponin, tannin, phenol, flavonoid, alkaloid, cardiac glycoside, terpenoid, protein, and steroid in the leave of *A. wilkesiana* as shown in Table 2.

Table 2. Phytochemical content of the leaves of *Acalypha wilkesiana*

Phytochemical	Status
Saponin	**+**
Tannin	**+**
Phenol	**++**
Flavonoid	**++**
Alkaloid	**+**
Cardiac glycoside	**++**
Terpenoid	++
Protein	++
Steroid	++

Table 3 shows the quantitative phytochemical constituents of the leaves of *A. wilkesiana*. The quantitative analysis of the leaves of *A. wilkesiana* reveals the presence of spartein and Ribalinidine which are alkaloids. It also reveals the presence of Naringenin, catechin, Epicatechin, Rutin and Kaempferol which are all flavonoids.

Tables 4, 5, 6 shows the effect of ethanol extract of the leaves *A. wilkesiana.*

3.1 Histopathology

The ability of A.W to ameliorate the effect of PARA was confirmed by the histological studies of the liver as shown in the plates below which supports the result presented in Tables 4, 5 and 6.

Normal control rats (group 1) showed normal hepatocytes (Plate 1) whereas the liver section PARA induced group had ballooning necrosis of the hepatocytes (Plate 2), this provides evidence of liver cell damage. The animals treated with A.W showed noticeable and improved hepatocyte as shown in Plates 3, 4, 5 and 6.

Table 3. Quantitative phytochemical constituents of *A. wilkesiana*

Components	Phytochemical	ug/ml	(%)
Spartein	Alkaloid	0.1221	0.0034
Phytate	Antioxidant	13.8305	0.3903
Naringenin	Flavonoid	0.3425	0.0041
Ribalinidine	Alkaloid	65.5711	1.850
Catechin	Flavonoid	815.9121	23.030
Epicatechin	Flavonoid	409.3268	11.553
Ritin	Flavonoid	1853.4909	52.317
Kaempferol	Flavonoid	384.5133	10.853

Table 4. Shows the effect of ethanol extract of the leaves *A. wilkesiana* in week 1

Treatment group	Total bilirubin (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Group 1	0.43±0.05^{c}	39.02±8.13^{ab}	230.33±12.45^{ac}	117.16±7.73^{b}
Group 2	0.46±0.96	75.50±11.62^{abc}	226.26±18.16^{bc}	226.40±38.54^{ac}
Group 3	0.43±0.02	68.43±2.02^{ac}	220.40±3.95^{ac}	131.23±14.84^{b}
Group 4	0.46±0.02	77.90±25.26^{a}	228.86±3.39	187.80±20.26^{a}
Group 5	0.43±0.03	78.36±3.37	239.80±4.35	268.30±22.15^{ac}
Group 6	0.41±0.06	82.43±0.90c	192.90±51.92^{b}	180.06±3.14^{b}

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c) are significantly different at p<0.05

Table 5. Shows the effect of ethanol extract of the leaves *A. wilkesiana* in week 2

Treatment group	Total bilirubin (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Group 1	0.43±0.05^{c}	39.02±8.13^{ab}	230.33±12.45^{ac}	117.16±7.73^{b}
Group 2	0.46±0.96	75.50±11.62^{abc}	226.26±18.16^{bc}	226.40±38.54^{ac}
Group 3	0.31±0.12^{a}	49.76±0.82	180.83±9.41	130.98±29.98
Group 4	0.39±0.67	72.33±2.93	221.23±25.74^{a}	154.80±14.58
Group 5	0.28±0.04^{a}	64.36±3.37	182.23±29.97	159.06±25.08
Group 6	0.28±0.02^{a}	79.03±0.90	184.33±18.22^{abc}	122.43±2.51

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c) are significantly different at p<0.05

Table 6. Shows the effect of ethanol extract of the leaves *A. wilkesiana* in week 3

Treatment group	Total bilirubin (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Group 1	0.43±0.05^{c}	39.02±8.13^{ab}	230.33±12.45^{ac}	117.16±7.73^{b}
Group 2	0.46±0.96	75.50±11.62^{abc}	226.26±18.16^{bc}	226.40±38.54^{ac}
Group 3	0.29±0.03^{a}	43.43±1.57^{bc}	136.33±31.55^{abc}	125.03±3.81^{bc}
Group 4	0.32±0.02^{a}	68.43±25.26^{a}	211.23±16.30	117.93±27^{b}
Group 5	0.39±0.32	57.90±3.51	164.23±44.66^{a}	117.36±10.01^{b}
Group 6	0.28±0.32^{a}	58.37±2.84	182.23±51.91^{ab}	162.26±41.42^{b}

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c) are significantly different at p<0.05

Plate 1 shows the liver histology of normal control (group 1) showing normal hepatocytes.

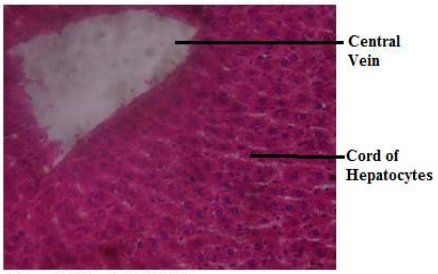

Plate 1. The liver histology of group 1 rats

Plate 2 shows the effect of PARA toxicity on the liver of the wistar albino rats while plate 3 to 6 below shows the effect of ethanol extract of *A. wilkesiana* on the liver histology of the wistar albino rats.

Plate 2 shows the effect of PARA induced hepatic injury on the liver histology of the wistar albino rats.

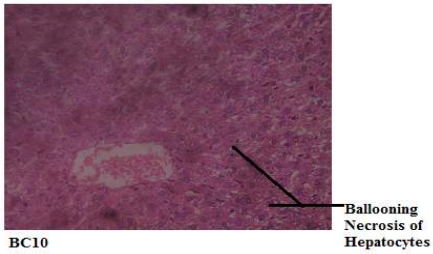

Plate 2. The liver histology of group 2 rats

Plate 3 shows the liver histology of wistar albino rats given 2000 mg/kg body weight of PARA and treated with 100 mg/kg body weight of ethanol extract of *A. wilkesiana.*

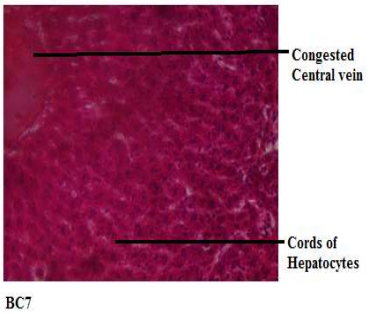

Plate 3. The liver histology of group 3 rats

Plate 4 shows the liver histology of wistar albino rats given 2000 mg/kg body weight of PARA and treated with 200 mg/kg body weight of ethanol extract of *A. wilkesiana.*

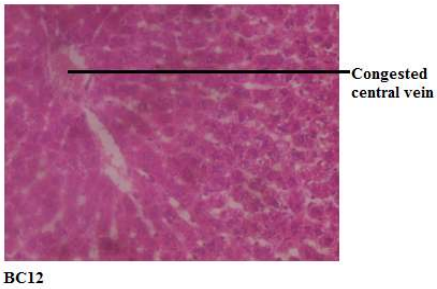

Plate 4. The liver histology of Group 4 rats

Plate 5 shows the liver histology of wistar albino rats given 2000 mg/kg body weight of PARA and treated with 300 mg/kg body weight of ethanol extract of *A. wilkesiana*.

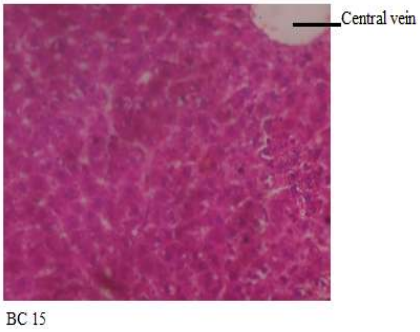

Plate 5. The liver histology of group 5 rats

Plate 6 shows the liver histology of wistar albino rats given 2000 mg/kg body weight of PARA and treated with standard drug silymarin.

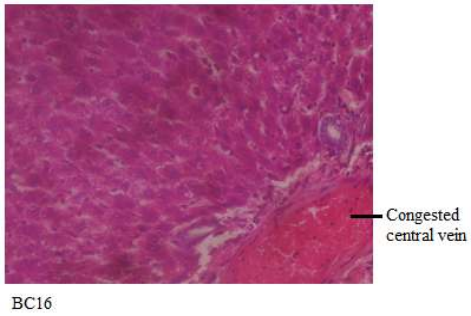

Plate 6. The liver histology of group 6 rats

3.2 Discussion

Paracetamol is an analgesic and antipyretic drug which is used in the treatment of pains, fever and headaches. Normally its metabolite known as N-acetyl-p-benzoquinon imine (NAPQI) is converted to cysteine and mercapturic acid by conjugating with the liver's glutathione. In the absence of this process, N-acetyl-p-benzoquinon imine remains in its toxic form in the liver. This toxic metabolite reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and acute hepatic necrosis thus liver damage [9]. The increase in the total bilirubin level and the liver enzyme markers shows the toxic effect of paracetamol when taken in over dose.

Different doses (100, 200 and 300 mg/kg) of the ethanol extract of the leaves of *A. wilkesiana* independently reduced the effect of paracetamol toxicity on the liver. The figures in the tables above shows the hepato-therapeutic potentials of the ethanol extract of the leaves of *A. wilkesiana.* The ethanol extract of the leaves of *A. wilkesiana* was able to reduce the activities of the liver enzymes alanine transaminase, aspartate transaminase and alkaline phosphatase and also the total bilirubin levels as shown in Tables 4, 5 and 6. This is a clear indication of the effect of ethanol extract of *A. wilkesiana* on paracetamol induced hepatic injury. The effect of the ethanol extract of *A. wilkesiana* on the liver enzymes is in agreement with the work of Ogbuehi et al. [1].

Plants have been used for so many years and in different countries for the treatment of different kinds of diseases. This is because of the different types of phytochemicals contained in these plants. Our research has shown that *A. wilkesiana* is a rich source of phytochemicals.

Flavonoids which are mostly present in the leaf extract of the plant have a lot of potentials in disease prevention and treatment. In line with other research flavonoids have protective and ameliorative effect against acetaminophen (paracetamol) induced hepatic injury [10,11,12,13]. The tannin present in the leaves of *A. wilkesiana* may also have contributed to the therapeutic effect of the plant. Ikewuchi et al. (2011) reported that *A. wilkesiana* is rich in tannin and tannic acid which are antioxidants involved in hepatoprotective effects. The hepatotherapeutic potentials of *A. wilkesiana* may also be as a result of its rich vitamin C content as reported by Ikewuchi and Ikewuchi [14]. The ability of *A. wilkesiana* to ameliorate liver damage caused by paracetamol toxicity may be due to the ability of its antioxidants to increase the liver's glutathione synthesis which was depleted as a result of paracetamol overdose.

4. CONCLUSION

In conclusion, the result of this research has shown that the ethanol leaves extract of *A. wilkesiana* has therapeutic effect on paracetamol induced hepatotoxicity. Its rich phytochemical content also supports the use of this plant for treatment of a wide range of infection in Western Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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