

Original Research Article

Ethanol extract of *Acalypha wilkesiana* leaves ameliorates paracetamol-induced hepatotoxicity in rats.

ABSTRACT

Objective: The effect of ethanol leaf extract of *Acalypha 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 orally. The extract was administered to paracetamol treated wistar albino rats at a dose of 100mg/kg, 200mg/kg and 300mg/kg body weight. Qualitative phytochemical analysis of the leaves of *Acalypha wilkesiana* showed that they are rich in flavonoids, phenols, tannins, terpenoids, cardiac glycosides, protein, alkaloids and steroids. The effect of the ethanol leaf extract of *Acalypha 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 *Acalypha wilkesiana* significantly ($p<0.05$) lowered the activities of alkaline phosphatase, alanine and aspartate transaminases.

Conclusion: Histopathological studies showed that ethanol leaf extract of *Acalypha wilkesiana* had a therapeutic effect on the liver of wistar albino rats with paracetamol induced hepatotoxicity

Key words: *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 borns. *Acalypha wilkesiana* has been reported to have anti-cancer, anti-malarial [12], anti-diabetic, anti-fungal, anti-microbial, anti-inflammatory and anti-pyretic effect [4]. Phytochemicals are plant chemicals which serve as antioxidants. Antioxidants inhibit the oxidation of free radicals that may cause cellular damage and cancer. Phytochemicals such as carotenoids acts as scavengers for free radical that damage the body tissues, Lycopene, a carotene which gives red colour to fruits (tomatoes, water melon) and vegetables has been linked to a lower risk of prostate cancer [1]. Studies have shown that carotenoids and flavonoids which are main found in colourful fruits and vegetables have potentials to reduced 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. This is due to depletion of the liver's glutathione which conjugates N-acetyl-para-benzoquinone imine to cysteine and mercapturic acid.

2.Material and method

2.1 Collection of plant sample and preparation of leave extract

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

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

36 **2.2 Determination of phytochemical content**

37 Twenty grams (20g) of the homogenized sample was mixed with 60g of anhydrous sodium
38 sulphate in agate mortar to absorb moisture. The homogenate was placed in a 500ml beaker,
39 300ml of n-hexane was added for extraction to be carried out using EPA 354° C method for
40 48h. Crude extract obtained was evaporated to dryness using a rotary vacuum evaporator at
41 40° C. The residue was transferred with n-hexane into a 5ml florisil column for clean-up.

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43 **2.3 Experimental design for hepatotherapeutic study**

44 Research was carried out according to the rules and regulations guiding the use of animals.
45 The animals were sorted into six groups with nine (9) animals each. The animals were
46 grouped as follows:

47 Group 1 Normal control, were allowed free access to water and food ad libitum without
48 treatment.

49 Groups 2: Hepatotoxic control received 2000mg/kg body weight of paracetamol but not
50 treated.

51 Group 3: Low dose group received 2000mg per kg body weight of paracetamol and treated
52 with 100 mg per kg body weight of ethanol extract of *Acalypha wilkesiana*.

53 Group 4: Medium dose group received 2000mg per kg body weight of paracetamol and
54 treated with 200 mg per kg body weight of ethanol extract of *Acalypha wilkesiana*.

55 Group 5: High dose group received 2000mg per kg body weight of paracetamol and treated
56 with 300 mg per kg body weight of the ethanol extract of *Acalypha wilkesiana*.

57 Group 6: Drug control: received 2000mg per kg body weight of paracetamol and treated with
58 standard drug Sylmarin (25mg per kg body weight).

59 Treatment commenced 48 hours after they received paracetamol. Mode of administration
60 was adopted from the work done by Ikewuchi [11]. Three (3) rats from each of the groups
61 were sacrificed at seven (7) days interval during treatment for three (3) weeks. The animals
62 were anaesthetized using chloroform. The animals while under anaesthesia were painlessly
63 sacrificed and the blood sample were collected into Heparin bottles for Chemistry test and
64 the liver was collected and preserved in 10% Formal-saline solution for Histological
65 examination.

66 **Determination of plasma hepatospecific enzyme**

67 The plasma activities of alanine and aspartate transaminases, and alkaline phosphatase were
68 determined using Mindray test kits. The activities of alanine and aspartate transaminases were
69 monitored at a wavelength of 540 nm while that of alkaline phosphatase was monitored at
70 a wavelength of 405 nm. The plasma total bilirubin, total protein and albumin concentration
71 were determined using mindray test kits. The plasma total bilirubin concentration was

72 measured at a wavelength of 578 nm, that of total protein was measured at 546nm while the
73 plasma albumin level was measured at 578nm.

74 **Histopathological study on the liver**

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

80 **Statistical analysis**

81 All data were subjected to statistical analysis. Values are reported as Mean $\pm$ standard error of
82 mean (SEM) while one way ANOVA was used to test for differences between treatment
83 groups using Statistical Package for Social Sciences (SPSS) version 16. The results were
84 considered significant at p -values of less than 0.05, that is, at 95% confidence level ($P<0.05$).

85 **3. Result and Discussion**

86 The preliminary qualitative screening revealed the presence of saponin, tannin, phenol,
87 flavonoid, alkaloid, cardiac glycoside, terpenoid, protein, and steroid in the leave of *Acalypha*
88 *wilkesiana* as shown in Table 1.1 below.

89 **Table 1.1 Phytochemical content of the leaves of *Acalypha wilkesiana***

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

Table 1.2 below shows the quantitative phytochemical constituents of the leaves of *Acalypha wilkesiana*. The quantitative analysis of the leaves of *Acalypha 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

Table 4.2: Quantitative phytochemical constituents of *Acalypha wilkesiana*

Component	phytochemical	ug/ml	(%)
Sparteina	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
Rutin	Flavonoid	1853.4909	52.317
Kaempferol	Flavonoid	384.5133	10.853

90 The effects of the ethanol leaf extract of *Acalypha wilkesiana* on the liver of wistar albino rats
 91 are shown in the tables below

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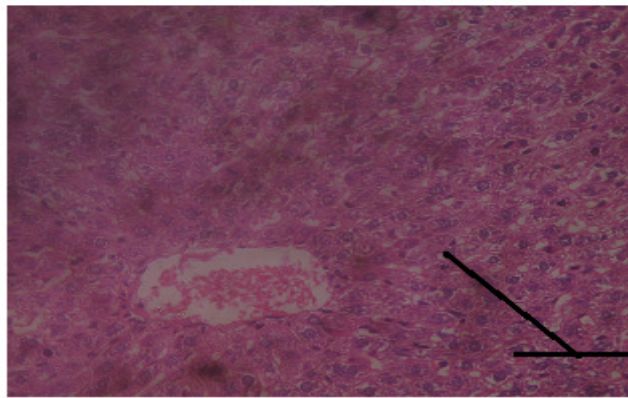
98 **Table 1** shows the effect of ethanol extract of the leaves *Acalypha wilkesiana*

Treatment GROUP	Total biliurubin	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control rats.	0.43±0.05 ^c	39.02±8.13 ^{ab}	230.33±12.45 ^{aC}	117.16±7.73 ^b
Positive control rats.	0.46±0.96	75.50±11.62 ^{abc}	226.26±18.16 ^{bc}	226.40±38.54 ^{ac}
Positive control rats on standard drug (syllimarin).	0.29±0.03 ^a	43.43±1.57 ^{bc}	136.33±31.55 ^{abc}	125.03±3.81 ^{bc}
Positive control rats on 100mg/kg body weight Of the plant extract.	0.32±0.02 ^a	68.43±25.26 ^a	211.23±16.30	117.93±27 ^b
Positive control rats on 200mg/kg body weight Of the plant extract.	0.39±0.32	57.90±3.51	164.23±44.66 ^a	117.36±10.01 ^b
Positive control rats on 300mg/kg body weight Of the plant extract	0.28±0.32 ^a	58.37±2.84	182.23±51.91 ^{ab}	162.26±41.42 ^b

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

101 **Plate 1 below shows the effect of paracetamol toxicity on the liver of the wistar albino**
 102 **rats while plate 2 to 5 below shows the effect of ethanol extract of *Acalypha wilkesiana***
 103 **on the liver histology of the wistar albino rats.**

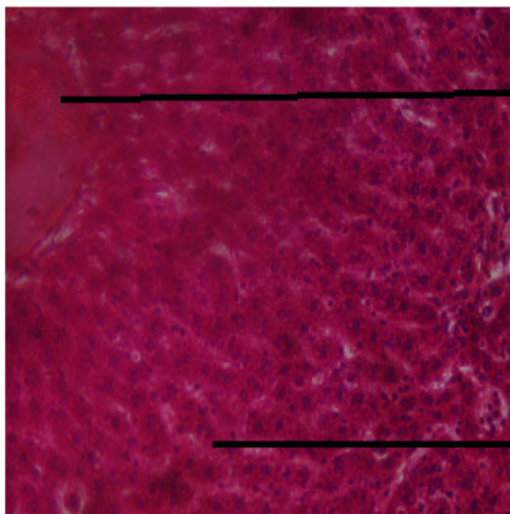
104 **Plate 1** below shows the effect of paracetamol induced hepatic injury on the liver histology
 105 of the wistar albino rats.



BC10

**Ballooning
Necrosis of
Hepatocytes**

Plate 2 below shows the liver histology of wistar albino rats given 2000mg/kg body weight of paracetamol and treated with 100mg/kg body weight of ethanol extract of *Acalypha wilkesiana*

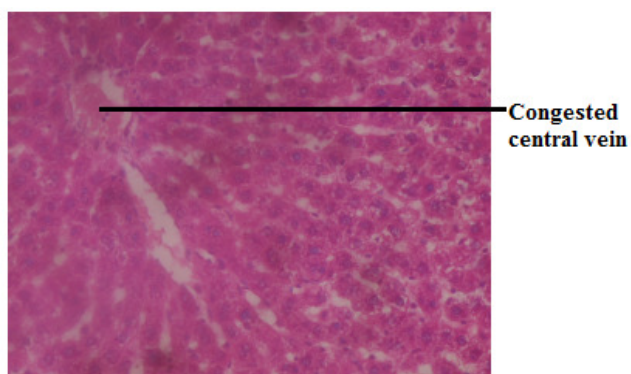


BC7

**Congested
Central vein**

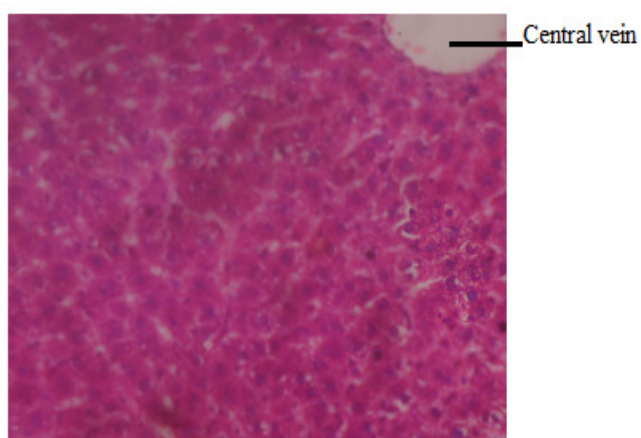
**Cords of
Hepatocytes**

117 Plate 3 below shows the liver histology of wistar albino rats given 2000mg/kg body weight of
118 paracetamol and treated with 200mg/kg body weight of ethanol extract of *Acalypha*
119 *wilkesiana*



120 **BC12**

121 **Plate 4** below shows the liver histology of wistar albino rats given 2000mg/kg body weight
122 of paracetamol and treated with 300mg/kg body weight of ethanol extract of *Acalypha*
123 *wilkesiana*.



124 **BC 15**

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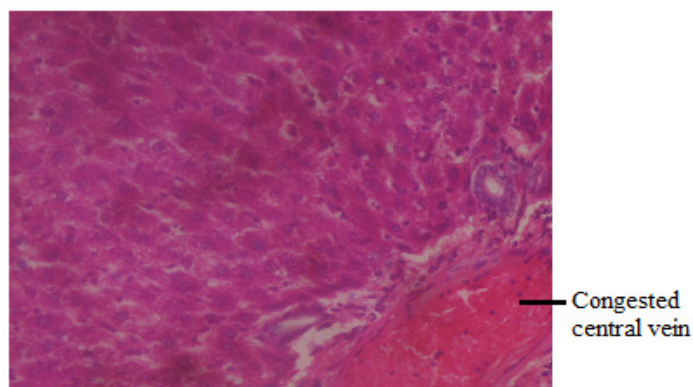
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132 Plate 5 below shows the liver histology of wistar albino rats given 2000mg/kg body weight of
133 paracetamol and treated with standard drug silymarin.



134 BC16

135 DISCUSSION

136 Paracetamol is an analgesic and antipyretic drug which is used to relief pain, fever and
137 headaches. Normally its metabolite known as N-acetyl-p-benzoquinon imine (NAPQI) is
138 converted to cysteine and mercapturic acid by conjugating with the liver's glutathione. In the
139 absence of this process, N-acetyl-p-benzoquinon imine remains in its toxic form in the liver.
140 This toxic metabolite reacts with cellular membrane molecules, resulting in widespread
141 hepatocyte damage and acute hepatic necrosis thus liver damage [8]. The increase in the total
142 bilirubin level and the liver enzyme markers shows the toxic effect of paracetamol when
143 taken in over dose.

144 Different doses (100, 200 and 300mg/kg) of the ethanol extract of the leaves of *Acalypha*
145 *wilkesiana* independently reduced the effect of paracetamol toxicity on the liver. The figures
146 in the table above shows the hepato-therapeutic potentials of the ethanol extract of the leaves
147 of *Acalypha wilkesiana*. The ethanol extract of the leaves of *Acalypha wilkesiana* was able to
148 reduce the activities of the liver enzymes alanine transaminase, aspartate transaminase and
149 alkaline phosphatase from 75.50 ± 11.62 (u/l), 226.26 ± 18.16 (u/l) and 226.40 ± 38.54 (u/l)
150 respectively to 57.90 ± 3.51 (u/l), 164.23 ± 44.66 (u/l) and 117.36 ± 10.01 (u/l) respectively. The
151 bilirubin levels was also reduced from 0.46 ± 0.96 to 0.28 ± 0.32 . This is a clear indication of
152 the effect of ethanol extract of *Acalypha wilkesiana* on paracetamol induced hepatic injury.
153 The effect of the ethanol extract of *Acalypha wilkesiana* on the liver enzymes is in agreement
154 with the work of Ogbuehi *et al.*, 2014 [12]. The therapeutic potential of this leave extract may
155 be as a result of its phytochemical content. Ikewuchi *et al.*, (2011) reported that *Acalypha*
156 *wilkesiana* is rich in tannin and tannic acid which are antioxidants involved in
157 hepatoprotective effects [11]. The hepatotherapeutic potentials of *Acalypha wilkesiana* may
158 also be as a result of its rich vitamin C content as reported by Ikewuchi and Ikewuchi [9].

159 Conclusion

160 The result of this research has shown that the ethanol extract of the leaves of *Acalypha*
161 *wilkesiana* has therapeutic effect on paracetamol induced hepatotoxicity. Its rich
162 phytochemical content also supports the use of this plant for treatment of a wide range of
163 infection in Western Nigeria

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