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Anti-inflammatory Assessment of Methanol Extract of *Acalypha ciliata* Leaves and It's Leucocyte Mobilization in Adult Wistar Rats

Ugwu Obiora Celestine [a,b*], Ali Ibeabuchi Jude [c], Asogwa Felix Keneolisa [a], Ukpabi-ugo Jacinta Chigozie [d], Omeh Romanus Chijioke [e], Okonkwo Raymond Maduabuchi [a], Ugwoke, Faith Ifeanyi [f], Offiah Raymond Ogbonna [g] and Anosike Chioma Assumpta [b]

[a] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria.
[b] Department of Biochemistry, Faculty of Biological Science University of Nigeria, Nsukka, Nigeria.
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani Enugu, Nigeria.
[d] College of Natural Science, Micheal Okpara University of Agriculture, Umudike, Nigeria.
[e] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani Enugu, Nigeria.
[f] Uburu Bioresources Biotechnology Development Agency, Abuja, Nigeria.
[g] Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Enugu State University of Science and Technology, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors UOC and ACA designed the study, performed the statistical analysis, wrote the protocol. Author UOC and UJC wrote the first draft of the manuscript. Authors ORC and ORM review the final drafted manuscript. Authors FKA and AIJ managed the analyses of the study. Author UFI and ORO managed the literature searches. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: Obiora.ugwu@esut.edu.ng;*

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ABSTRACT

Aim: The study's objective was to examine the anti-inflammatory activity and leucocyte mobilization of a methanol extract of leaves from *Acalypha ciliata* in adult Wistar rats.
Study Design: Egg albumin-induced edema was used for anti-inflammatory test and leucocyte mobilization test was carried out to check for total leucocyte cout and differentials.
Place and Duration of the Study: This original study was conducted between January and June 2013 at the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka.
Methology: The pulverized leaf of *Acalypha ciliata* was extracted using cold maceration, and the Treas and Evans technique of phytochemical screening was used. The anti-inflammatory study was conducted using the method of Winter et al., and the acute toxicity study was assessed using Lorke's method. ANOVA was used to statistically examine the collected data.
Results: Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, soluble carbohydrates, phenols, glycosides, saponins, terpenoids and steroids. Flavonoids, Alkaloids, and Soluble carbohydrates appeared in abundant concentration (2199.76±10.81, 2141.6±7.583, 913±21.06). Acute toxicity tests showed no toxicity and mortality at doses up to 5000 $mgkg^{-1}$. Anti-inflammatory study revealed that group treated with 100, 200 and 400 mg/kg b.w of extract significantly (P =0.05) and in a dose dependant manner decreased in TLC when compared with normal control. Moreover, the group treated with 100,200,400 mg/kg b.w of extract and reference drug(Indometacin) mobilized the leucocyte at the rate of 36, 22, 12 and 62 % respectively. A significant (P = 0.05) reduction in the mean paw oedema was observed for all the treatment groups from 1 hour to 5 hours when compared to the toxic group.
Conclusion: As a result of this study's findings, it can be concluded that methanol extract of the *Acalypha ciliata* leaf has anti-inflammatory characteristics. High dosages of the extract resulted in a better decrease of oedema and an increase in leukocyte mobilization in response to agar suspension than low doses. Many anti-inflammatory herbs and substances alter inflammatory reactions by hastening the breakdown of or reversing the effect of the inflammatory mediators. The plant's anti-inflammatory properties could potentially result from the interaction of different photochemical substances present. The findings suggest that, if used, the plant may serve as a source of anti-inflammatory compounds.

Keywords: Acalypha ciliate; anti-inflammatory; paw oedema; leukocyte migration

1. INTRODUCTION

A medicinal plant can be defined as a species of plant with one or more bioactive molecule, used in folk medicine for treating diseases in human and animals [1]. Medicinal plants have been used since ancient civilization to treat various diseases [2].The vascular and cellular response of living tissues to injury is known as inflammation [3]. Swelling, redness, heat, pain, and a loss of physical function are some of its symptoms [4]. The inflammatory process works to eliminate, weaken, or wall-off the harmful substance as well as any potentially harmed tissue cells. In other words, a complicated chain of circumstances is set in motion in an effort to repair and rebuild the injured tissue. In addition, inflammation benefits the host [5]. Infections would go untreated, burns would not heal, and wounds would stay infected open sores without inflammation [6]. But, under certain circumstances, inflammation can become dangerous. The vascular tissues' complex biochemical response to a harmful stimulus is inflammation. This response is assumed to be a defense mechanism the body employs to eliminate unwelcome stimuli, which can include viruses, damaged cells, infections, or irritants [7]. The activation of immune cells and the release of chemical mediators from wounded cells or tissues at the injury site speed up this non-specific defensive response [8,9]. Serotonin,

prostaglandins, kinin, histamine, and other molecular mediators all work together to promote capillary permeability and vasodilation [10]. Acute inflammation and chronic inflammation are the two categories into which inflammation can be divided. Acute inflammation is a rapid response that lasts for a few minutes to several hours. Migration and the leaking of plasma proteins or fluids from the blood to the site of damage are symptoms of acute inflammation [11]. Chronic inflammation, which is marked by a significant immune cell infiltration at the site of damage, can develop from persistent inflammation brought on by protracted exposure to damaging stimuli [12]. *Acalypha ciliata* belongs to the family of *Euphorbiaceae* [13]. The leaves are arranged spirally, simple and stipules are linear. The plant is monoecious and the stem is shortly and hairy. The plant is considered by traditional practitioners to possess expectorant, purgative, emetic, gastrointestinal irritative and diuretic properties. As a result of the acclaimed pharmacological importance of *Acalypha ciliata* leaves, this study was therefore aimed at investigating its anti-inflammatory properties as an insight into the use of the plant as a potential source of new anti-inflammatory drug.

2. MATERIALS AND METHODS

2.1 Collection and Preparations of Extract

Thefresh leaves of *Acalypha ciliate* was collected from Opi in Nsukka town, Enugu state of Nigeria. This plant was identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) research center Nsukka, Nigeria. Fresh *Acalypha Ciliata* leaves were minced into tiny pieces, allowed to air dry for two weeks, and then mechanically ground into a coarse powder. A rotary evaporator (IKA, Germany) was used to concentrate the filtrate at an ideal temperature of 55-60^{0C} after the ground plant (600 g) had been macerated in methanol for 72 hours.

2.2 Laboratory Animals

Swiss albino mice (28-30 g) were used for toxicity testing, and adult male Wistar rats (120–190 g) from the department of veterinary medicine at the University of Nigeria Nsukka were employed for in-vitro anti-inflammatory activities. The animals were housed in metal steel cages and acclimated in the lab for seven days prior to the trials. Regular grower's mash rat pellets and water ad lib were given to the rats (Grand Cereals LTD, Enugu, Nigeria).

2.3 Drugs, Reagents and Chemicals

Analytical grade chemicals from Sigma Aldrich in the United States, British Drug House (BDH) in England, Burgoyne in India, Harkin and Williams in England, Qualikems in India, Fluka in Germany, and May and Baker in England were all used in this study. The assays were conducted with retail kits and goods from Teco (TC) and Randox in the United States. Standard anti-inflammatory medications Indomethacin, Asprin, and Prednisolone (10 mg) were purchased from Elofex Pharmaceutical and Drug Stores in Enugu, Enugu State, Nigeria, a reputable pharmacy.

2.4 Phytochemical Analysis

Using the methods described by Harbone et al.[14], numerous quantitative chemical assays were performed to assess the phytochemical makeup of the crude extract.

2.5 Research on Acute Toxicity

The acute toxicity of the crude extract was investigated, and the median lethal dose (LD50) was estimated using [15] with some modifications to specify the lethal dose range. A total of 18 Swiss albino mice were used in the study, and they were given access to water but not food for 18 hours. Each group received MEACL at different dose levels (10, 100, and 1000 mg/kg for phase one and 1600, 2900, and 5000 mg/kg for phase two) and was divided into three (3) groups of three mice each. The animals were observed for signs of anxiety, lethargy, lack of coordination, mortality, and behavioral changes throughout the course of the following 24 hours.

2.6 Experimental Design

Twenty-five (25) male Wistar albino rats were used in the investigation. The rats were placed randomly in each groups of five (5) animals each and they received the following cares:

Group1: Toxic group
Group 2: Indomethacin 10 mg/kg body weight was administered (standard drug)
Group 3: MEACL 100 mg/kg body weight was administered.
Group 4: MEACL 200 mg/kg body weight was administered.
Group 5: MEACL 400 mg/kg body weight was administered.

2.7 Egg Albumin- Induced Oedema in Rats

It was done using rat edema [16]. As a model for acute inflammation, the volume of the right hind paw increased after receiving a sub plantar injection of fresh egg albumin. Twenty (25) mature male Wistar rats (120–190 g) were divided into five groups of five rats each for the plant extract. They went without food and water for 18 hours prior to the experiment. Water restriction was used to ensure equal hydration and reduce variation in the edematous response [17]. To groups III, IV, and V of the rats, respectively, different extract doses (100, 200, and 400 mg/kg) suspended in 3% Tween 80 were given intraperitoneally. The vehicle's corresponding amount, or 3% Tween 80, was given to the control group(1) and and group (II) was administered 10mg/kg indomethacin. After treatment, the right hind paw's subplanter surface was injected with 0.1 ml of undiluted fresh egg albumin (a philogistic substance) to cause inflammation. It was discovered that this therapy resulted in paw swelling, which remained at a same level of oedema for 3 hours. Rats' right hind paw volumes were measured using the volume displacement method at zero time (before the experiment) and at a 1-hour interval. The results were compared to the volume displacement before injecting the philogistic drug and the volume displacement of the injected paw at zero time (vt - vo). Using the relation [10], the percent inhibition of oedema was also determined for each dose;

$$\% \text{ inhibition of Oedema} = 1 - \left(\frac{a-x}{b-y}\right) \times 100$$

Where

a = mean paw volume of treated rats after egg albumin injection
x = mean paw volume of treated rats before egg albumin injection
b = mean paw volume of control rats after egg albumin injection
y = mean paw volume of control rats before egg albumin injection

2.8 Leucocytes Mobilization Test in Rats

Using the method described by Winter et al. [16], it was determined whether the methanol extract *of Acalypha ciliata* leaves affected in vivo leukocyte mobilization brought on by an inflammatory stimulus. Twenty-five (25) male adult Wistar rats (weighing between 120 and 190 g) were utilized in the experiment, five groups of five rats each. The extract was given to groups III, IV, and V in varying doses (100, 200, and 400 mg/kg), whereas groups I and II received 3% tween 80 and indomethacin (10 mg/kg), respectively. Each animal in the corresponding groups (n=5) received an intra-peritoneal injection of 0.5 ml of a 3% w/v agar suspension in normal saline three hours after receiving oral doses of the extracts, tween 80, and reference drugs. The animals were killed four hours later, and the peritoneal cavities were cleaned with 5 ml of a 5% EDTA in phosphate buffered saline solution (PBS). The peritoneal fluid was recuperate and both total and differential leukocyte counts (TLC and DLC) were carried out on the perfusates using manual cell counter after staining with Wright's stain. The percent mobilization of leukocytes was calculated using the formula:

$$\% \text{ Leukocyte mobilization (\% L.M)} = \left(1 - \left(\frac{T}{C}\right)\right) x100$$

Where T and C represent the leukocyte count of the treated and control groups respectively.

2.9 Statistical Analysis

All results obtained were expressed as mean ± SEM. Data obtained were analyzed by One-way analysis of variance (ANOVA) and was subjected to Turkey Post-hoc test using Graph Pad Prism Version 8.3 (GraphPad Software, San Diego, CA, USA) for multiple comparisons. Differences between means were considered significant at $P < 0.05$.

3. RESULTS

3.1 Percentage Yield of Crude Extract

The percentage yield of the crude extract was obtained to be appreciably 28.70%.

$$\%Yield = \frac{weight\ of\ crude\ extract}{wieght\ of\ pulverized\ coarse\ powder\ soaked} \times \frac{100}{1}$$

3.2 Phytochemical Screening

Flavonoids, alkaloids, phenols, glycosides, saponins, terpenoids, and steroids were found, according to the results of the phytochemical screening**.** saponins, glycosides and steroid appeared in low concentration (1.525±0.025,

0.45±0.099, 96.77±3.7), alkaloids, tannins and reducing sugar appeared in moderate concentration (642.00±215.7, 867.70±8.6, 432.64±0.004), whereas, Flavonoids, Phenols, and terpenoids appeared in abundant concentration (4264.00 ±360.2,14065.00 ±538.4, 5484.00±30.4) respectively.

3.3 Acute Toxicity Studies

The results of acute toxicity testing in mice was carried using the method described by (Lorke, 1983) showed that the Methanolic extract of *Acalypha ciliata* leaves was not toxic and there was no sign of behavioral changes up to a dose of 5000 $mgkg^{-1}$ body weight.

3.4 Effect of Methanol Extract of *Acalypha ciliata* Leaves (MEACL) on Albumin Induced Rat Paw Edema

The impact of MEACL on rat paw edema caused by egg albumin is shown in Table 3. According to the findings, egg albumin-induced edema in the rat paw was sustained over a period of 5 hours with a mean paw edema and a percentage inhibition. From one hour to five hours after treatment, all treatment groups showed a significant (p = 0.05) decrease in mean paw edema as compared to the toxic group. The mean paw edema of the rats in the control group did not decrease significantly (p=0.05) at any of the several time points. Animals given progressively higher doses of the extract plus indomethacin experienced a marked reduction in paw size over time.

The results depicted in Table 2 showed that group treated with 100, 200 and 400 mg/kg b.w of extract significantly (P = 0.05) and in a dose dependant manner decreased TLC when compared with normal control. Moreover, the group treated with 100,200,400 mg/kg b.w of extract and reference drug (Indometacin) mobilized the leucocyte at the rate of 36, 22, 12 and 62 % respectively.

The control group's total leucocyte count was the highest (5513 ± 539.10) and its proportion of migrating neutrophils was the highest (35.5%). The leucocytes that were most active were lymphocytes, and Basophils were barely mobilized.

Table 1. Quantitative phytochemical constituents of methanol extracts o*f Acalypha ciliata* leaves

Phytochemical	Qualitative remarks	Concentration (mg/100g)
Constituents		
Flavonoid	+ ++	4264.00 ±360.2
Terpenoid	+++	5484.00±30.4
Phenols	++ +	14065.00 ±538.4
Tannin	++	867.70±8.6
Alkaloids	++	642.00±215.7
Reducing sugar	++	432.64±0.004
Carbohydrates	++	913±21.06
Cardiac Glycosides	+	199.38±0.122
Saponin	+	1.525±0.025
Glycosides	+	0.45±0.099
Steroids	+	96.77±3.7

Table 2. LD_{50} of methanolic extract of *Acalypha ciliata* leaves

Groups	Dose of extract (mg/kg. b.w.)	Mortality	Behavioral changes
Phase 1			
Group 1	10	0/3	Nil
Group 2	100	0/3	Nil
Group 3	1000	0/3	Nil
Phase II			
Group 1	1600	0/3	Nil
Group 2	2900	0/3	Nil
Group 3	5000	0/3	Nil

Table 3. Effect of methanol extract of *Acalypha ciliata* leaves (MEACL) on egg albumin induced rat paw oedema

Groups	paw volume (oedema) (ml)				
	1hr	2hr	3hr	4hr	5hr
Control	0.77 ± 0.21	0.73 ± 0.17	0.72 ± 0.17	0.58 ± 0.13	0.55 ± 0.10
10mg/kg	0.67 ± 0.11	0.50 ± 0.08	0.32 ± 0.05	0.15 ± 0.05	0.10 ± 0.00
Indomethacin	(12.98)	(31.51)	(55.55)	(74.13)	(81.81)
Extract	0.75 ± 0.13	0.70 ± 0.28	0.70± 0.12	0.50 ± 0.23	0.42 ± 0.17
100mg/kg	(2.6)	(4.11)	(2.78)	(13.8)	(23.6)
Extract	80.75 ± 0.24	0.65 ± 0.19	0.52 ± 0.17	0.35 ± 0.17	0.17 ± 0.11
(200mg/kg)	(2.6)	(10.95)	(27.78)	(39.66)	(69.09)
Extract	0.70 ± 0.00	0.57 ± 0.15	0.37 ± 0.15	0.35 ± 0.17	0.22 ± 0.15
(400mg/kg)	(9.09)	(21.91)	(48.61)	(39.66)	(60.18)

**Reduction in oedema significant at p<0.05 compared to control, **=p<0.01, ***=p<0.001*
Results are expressed in means ± SD. (n = 5)
Mean values having different upper case letters as superscripts are considered significant (p = 0.05) down the group

Table 4. Effect of methanol extract of *Acalypha ciliata* leaves on agar induced *In vivo* leukocyte mobilization

Treatment	Dose (mg/kg)	TLC Cell (mm^3)	% Mobilization	Differential Leucocyte Mobilization (%)			
				Neurophils	Lymphocyte	Basophil	Monocyte
Control		5513 ± 539.10	-	40	64	0	1.25
Indomethacin	10	*2100 ± 16.03	62	34.5	65.5	0	1
Extract	100	***3525 ± 689.81	36	29.5	70	0	0
Extract	200	***4325 ± 170.78	22	35.5	59.5	0	0.5
Extract	400	*4850± 267.71	12	28.75	70.25	0	1

4. DISCUSION

The anti-inflammatory actions of the MEACL were determined in the current investigation using in-vivo and in-vitro anti-inflammatory based assays. According to Table 1 of the quantitative phytochemical study, the plant includes different levels of tannins, phenols, flavonoids, alkaloids, terpenoids, glycosides, steroids, and saponins. High concentrations of flavonoids found in plants have been reported to have the ability to scavenge free radicals and to alter the generation of the cyclooxygenases (COX-1 and COX-2) involved in prostaglandin synthesis [18]. These bioactive components are assumed to be the cause of the anti-inflammatory activities of some therapeutic plants [19], via a number of methods, such as the suppression of transcription factors and regulatory enzymes, flavanoids produce anti-inflammatory effects. These factors are crucial in the regulation of inflammation-related mediators [20]. Studies on acute toxicity have revealed that oral doses of MEACL have an excellent safety profile. Animals tolerated the plant extract up to 5000 mg/kg without experiencing any fatalities, as demonstrated in Table 2. Table 3 displays the effect of MEACL on rat paw edema brought on by egg albumin. The extract may prevent the release of histamine and serotonin because of its capacity to prevent the early stages of edema. Acute inflammation is characterized by vasodilatioin, the exudation of protein-rich fluid (plasma) and leucocytes migration into the site of injury [21]. The egg albumin model is characterized by production of edema. In the relatively rapid early phase (1 to 2hrs), edema formation is mediated by histamine and serotonin and the later phase (3 to 5hrs), kinin and prostaglandins contribute to the edema [22]. The egg albumin induced edema is useful in detecting activity in acute inflammation. There was decrease in leucocyte mobilization upon treatment with 100,200 and 400mg/kg B.W of extract. The drugs likely suppressed the inflammatory mediators' ability to proliferate and cause an increase in the production of leucocytes that could migrate to the area where the various leucocytes mobilized, lympocytes is the most prevalent. The agar suspension was able to cause an injury that was responded to by the drugs. Lympocytes and other phagocytic cells proliferate more frequently during injury, which directly contributes to the substantial mobilization of leukocytes to the site of an injury [23]. They cause inflammation, break down phagocytosed material, and damage the extracellular matrix [24]. The use of indomethacin in this study reduced the mobilization of leukocytes, which is consistent with the findings of Tukur et al. [25], which showed that indomethacin in large doses suppressed the accumulation of leukocytes.

5. CONCLUSION

In conclusion, the results from this research work revealed that methanol extract of *Acalypha ciliata* leave possesses anti-inflammatory properties and high dose of the extract yielded a better reduction in edema and increases mobilization in leukocyte in response to agar suspension than in lower doses. Many anti-inflammatory herbs and substances alter inflammatory reactions by hastening the breakdown of or reversing the effect of the inflammatory mediators. The plant's anti-inflammatory properties could potentially result from the interaction of different photochemical substances present. The findings suggest that, if used, the plant may serve as a source of anti-inflammatory compounds.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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