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# *Eleusine indica* Linn, Baertin (*Poaceae*) Ethanol Leaf Extract and Its Ethyl Acetate Fraction Display Potential Anti-inflammatory Activities

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author PAA designed the study and carried out the statistical analysis. Author AOE performed the experiments and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Objective:** Inflammation is the underlying cause of most of the chronic diseases that occur with aging. Although many drugs are available for the management of inflammatory disorders and their symptoms, most of these drugs possess serious adverse effects that limit their usefulness. This has encouraged the unending search for potent anti-inflammatory drugs from plant sources as alternatives to conventional drug treatment of inflammation. This study investigated the anti-inflammatory activities of the ethanol leaf extract of *E. indica* and the ethylacetate fraction in rodents.
**Materials and Methods:** The leaves were extracted with ethanol by cold maceration and the extract was fractionated with n-hexane, ethylacetate, butanol and water. The oral acute toxicity ($LD_{50}$) of the extract and the phytochemical constituents of the extract and the fractions were determined. The anti-inflammatory activities of the ethanol extract (EE) and ethylacetate fraction (ETF) and their possible mechanisms of actions were investigated.

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**Results:** The oral $LD_{50}$ of the extract was above 5000 mg/kg. Both the EE and ETF displayed dose-dependent inhibition of the rat paw edema, with ETF producing between 48-54% edema inhibition. Xylene-induced topical edema was significantly ($p < 0.05$) reduced by both the EE and ETF, with ETF causing between 48 and 65% inhibition. The EE and ETF preserved the integrity of gastric mucosa. Their average ulcer index (1.37±0.02) was significantly lower than that of indomethacin (5.20±0.23). Pre-treatment with the EE and ETF significantly ($p < 0.05$) reduced leucocyte migration, especially the neutrophils. Both heat- and hypotonicity-induced hemolysis of RBC membrane were remarkably inhibited.
**Conclusion:** The mechanisms of the anti-inflammatory activity may involve among others inhibition of leukocyte migration and membrane stabilization.

*Keywords: Eleusine indica; ethanol extract; ethylacetate fraction; anti-inflammatory; leucocyte migration; rodents.*

## 1. INTRODUCTION

Inflammatory reactilons underline a vast variety of human diseases [1]. Inflammation is a normal protective response to tissue injury. It is the body's effort to inactivate or destroy invading organism, remove irritants, and set the stage for tissue repair [2]. Common signs and symptoms of inflammation includes, pain, redness, bruising, fever, chills, stuffy nose and head, joint swelling and stiffness, tender muscle parts, fluid retention and loss of joint function [3]. It is also associated with flu- like symptoms, fatigue, headache, loss of appetite and muscle weakness [4].

Inflammation may be caused by microbial infection [5], physical agents [6], irritants and corrosives chemicals [7], tissue necrosis [8] and hypersensitivity reactions [9]. The immune system is often involved in inflammatory disorders, demonstrated in both allergic reactions and some myopathies. Inappropriate activation of the immune system can result in inflammation leading to rheumatoid arthritis [10]. Non-immune diseases with etiological origins in inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease [11]. A large variety of proteins are involved in inflammation, and any one of them is open to genetic mutation which impairs or otherwise deregulates the normal function and expression of that protein [12].

Inflammation occurs in orderly sequence involving the initiation of inflammatory process by a foreign substance or physical injury, recruitment and chemo-attraction of the inflammatory cells and activation of these cells to release inflammatory mediators capable of damaging or killing invading microbes or tumour [13].

The cardinal signs of inflammation include hot inflamed site due to increase in blood flow towards the region, redness, and swelling due to vascular permeability, pain caused by the activation and sensitization of primary afferent neurons and lasting loss of function and mobility (14]. Pain and fever are the most common complaints associated with inflammation. The non steroidal anti-inflammatory drugs (NSAIDs) are widely used in the management of pain and other inflammatory conditions. They however do not cure and remove the underlying causes of the disease but, only modify the inflammatory responses to the disease [14]. The plethoras of adverse effects associated with these agents have led to a search for effective and safer anti-inflammatory drugs from plant sources as alternatives to conventional drug treatment [15].

In the south eastern Nigeria, a good number of plants are used traditionally to treat inflammatory conditions especially rheumatoid arthritis. *Eleusine indica* Linn (Poaceace) commonly known as wire grass, goose grass or crowfoot grass is one of the popular traditional anti-inflammatory folk remedy. It is locally known as "Ese" in the south eastern Nigeria. In this region, the leaves of *E. indica* are applied externally to open wounds to arrest bleeding. A poultice of the leaves is applied to sprains and back pains, while a decoction of the macerated leaves is used to treat skin rashes, painful swelling, fevers and asthma. Few biological studies on the plant reported activities such as anti-diabetic [16], anti-inflammatory [17], anti-plasmodial [16,18], antioxidant and antibacterial [19] and analgesia [20]. The anti-inflammatory activity of *E. indica* leaves and the possible underlying mechanisms of action were investigated in this study.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals, Reagents, and Drugs

All the chemicals and reagents used for the experiments (70% ethanol, ethylacetate,

chloroform, xylene, Tween 80, 3% w/v agar, aspirin indomethacin, acetic acid, EDTA, gentian violet, prednisolone,) were of analytical grade and products of Sigma Aldrich, Germany.

## 2.2 Animals

Swiss albino rats (150 – 180 g) and mice (25 – 30 g) of both sexes were obtained from the Animal Facility Center of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University Awka. The animals were maintained at laboratory animal conditions and were allowed free access to food and water.

## 2.3 Collection and Preparation of Plant Material

Fresh leaves of *Eleusine indica* were collected from Uke in Idemili Local Government Area Anambra State, Nigeria in October and November 2014. The collection was authenticated by Mr A. Ozioko of International Centre for Drug Development (Inter-CEDD) Nsukka, Enugu State. The leaves were carefully separated from the woody part, cut into small pieces, sun-dried for 7 days and pulverized using a milling machine.

## 2.4 Extraction and Fractionation

About 1.5 kg of the pulverised leaves was extracted by cold maceration using 70% ethanol for 48 hr with intermittent shaking. The extract was filtered using Whitman filter paper and the filtrate concentrated using rotary evaporator at 40°C. Part of the concentrated extract was subjected to liquid-liquid chromatographic fractionation using solvents of different polarity to obtain n-hexane, ethylacetate, butanol and aqueous fractions. The resulting fractions were concentrated using rotary evaporator at 40°C except water fraction that was concentrated with freeze dryer. Preliminary studies indicated ethanol extract (EE) and the ethylacetate fraction (ETF) as the most effective, and were thus subjected to further studies.

## 2.5 Acute Toxicity ($LD_{50}$) and Phytochemical Studies

The oral acute toxicity ($LD_{50}$) of the ethanol extract (EE) in rats was determined [21], while the phytochemical analysis of both EE and the fractions were performed using standard methods [22,23].

## 2.6 Anti-inflammatory Evaluations

Evaluation of the anti-inflammatory potentials of the extract and fraction were done using acute systemic inflammatory model (egg-albumin-induced edema) and topical (xylene-induced edema) model. This would indicate if the extract and fraction possess both systemic and topical anti-inflammatory activty

### 2.6.1 Effect on egg albumin induced rat paw edema

Thirty (30) minutes before sub planter injection of 0.1 ml freshly undiluted egg albumin in the rats' right paw, oral administrations of 200, 400, and 600 mg/kg of EE or ETF were given to 3 different groups of rats (n=6). Aspirin (100 mg/kg) and 10% Tween 80 (10 ml/kg) was given to the positive and negative control groups respectively. Paw volumes (edema) were measured by water displacement methods [24]. Edema volume was measured before and at 1, 2, 3, 4, 5 and 6 hr after sub planter injection of egg albumin. The anti-inflammatory effect was expressed as percent inhibition of edema in the treated animals in comparison with the vehicle treated animals [25]. The percent inhibition of edema was calculated using the formula [25]:

$$(Vc-Vt)/Vc \times 100$$

Where Vc is the mean edema (paw volume) of the control and Vt the mean edema of the treated, and mean edema is the mean difference of paw volume displaced at time t and that of basal paw volume at time zero.

### 2.6.2 Effect on topical edema induced by xylene in the mouse ear

The effect of the EE and ETF on acute topical inflammation was evaluated [26]. Eight groups of adult Swiss albino mice of either sex (n=6) were used for the study. The extract or fraction 200, 400 and 600 µg was applied on the anterior surface of the right ear of 6 groups of mice. Topical inflammation was immediately induced on the posterior surface of the same ear by application of xylene (0.05 ml). Control groups were given either the vehicle (Tween 80) or indomethacin 200 µg/ear. Two hours after induction of inflammation, the mice were sacrificed by overdose of chloroform anesthesia and both ears removed. Circular sections (7 mm diameter) of both the right (treated) and left

(untreated) ears were punch out using a cork borer and weighed. The values obtained from the left ears were subtracted from that of the right ears. Edema was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was evaluated as percentage edema inhibition in the treated animals relative to control animals using the relation [27]:

Percentage edema reduction (%) = [1 - (Rt – Lt/ Rc –Lc)] X 100]

Where

*R*t = mean weight of right ear plug of treated animals;
*L*t = mean weight of left ear plug of treated animals;
*R*c = mean weight of right ear plug of control animals;
*L*c = mean weight of left earplug of control animals.

## 2.7 Mechanisms of Anti-inflammatory Activity

### 2.7.1 Ulcerogenic effects in rats

This study was performed as described by Cashin et al. [28]. Adult Swiss albino rats were fasted for 24 hr, and doses of the EE or ETF (200, 400 and 600 mg/kg) were administered orally to treatment groups (n=6). Control animals were giving either indomethacin 40 mg/kg or 10% Tween 80 (10 ml/kg). Three hours after drug administration, animals were sacrificed with ether. The stomachs were removed and cut along the greater curvature to expose the mucosal surface. The mucosa was washed with normal saline and observed with magnifying lens (x10). Injury to the mucosa was scored 0-4 on an arbitrary scale; 0 = normal coloured stomach, 0.5 = Red coloration, 1= one or two spot ulcer, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions/perforation [28].

### 2.7.2 *In vivo* leucocytes migration test

The effect of the EE and ETF on cell migration *in vivo* was evaluated in albino rats using the method described by Ribeiro et al. [29]. One hour after oral administration of 200, 400 and 600 mg/kg of the EE or ETF, the animals were given intraperitoneal injection of 1 ml of 3%, w/v agar suspension in normal saline. Four hours later, they were sacrificed and the peritoneal cavities washed with 5 ml of phosphate buffer saline containing 0.5 ml of 10% EDTA. The peritoneal fluid was recovered and the total and differential leucocytes counts (TLC and DLC) were performed on the perfusates.

### 2.7.3 Determination of total leucocytes count

A 1:20 dilution of the peritoneal fluid in the diluting fluid (2% acetic acid tinged with gentian violet) was prepared by adding 0.02 ml of peritoneal fluid to 0.38 ml of the diluted fluid. The Neubauer counting chamber was charged with the well-mixed diluted peritoneal fluid. The first 3-5 drops were discarded before charging the chamber. The cells were allowed to settle in a moist chamber for 3-5 minutes. Using 10x objective of the microscope, four large corner squares were located and the total numbers of white cells in the four large corner squares were counted using a manual cell counter after staining with Wright's stain.

### 2.7.4 Differential leucocytes count

The staining procedure is the same with the total leucocytes count, however, the cells were differentiated based on their morphological variations and slight difference in stains.

### 2.7.5 Membrane stabilization effects

Fresh whole human blood (5 ml) was collected from healthy human volunteers that had not taken any medications within the past two weeks. The blood was transferred to EDTA centrifuge tube. The tubes were centrifuged at 2000 rpm for 5 min, and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (pH 7.4), of the following composition/L of distilled water [30]; NaCl (9 g), $NaH_2PO_4$ (0.2 g) and $Na_2HPO_4$ (1.15 g).

### 2.7.6 Heat-induced hemolysis

The isotonic buffer solution (5 ml) each containing 200 400 and 600 µg/ml of the EE or ETF was put in four set of centrifuge tubes. Control tubes contained 5 ml Tween 80 or 5 ml of 200 µg/ml of prednisolone. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of each sample was incubated at 54°C for 20 min in a regulated water

bath. The other pair was maintained at 0-4°C in a freezer for 20 min. At the end of the incubation, the reaction mixture was centrifuged at 1000 rpm for 3 min and the absorbance of the supernatant measured spectrophotometrically at 540 nm using Spectronic 21D (Milton Roy) Spectrophtometer [30]. The percentage inhibition of hemolysis was calculated as follows;

Inhibition of hemolysis (%) = [1- (OD2-OD1/OD3-OD1)] x 100

OD1 = absorbance of test sample unheated
OD2 = absorbance of test sample heated
OD3 = absorbance of control sample heated

#### 2.7.7 Hypotonicity-induced hemolysis

The hypotonic solution (distilled water, 5 ml) containing 200, 400 and 600 µg/ml of EE or ETF was arranged in two pairs. Control tubes contained 5 ml of distilled water or 200 µg/ml indomethacin. Erythrocyte suspension (0.005 ml) was added to each tube and after gentle mixing, the mixture was incubated for 1hr at room temperature (30°C). At the end of the incubation, the reaction mixture was centrifuged at 1000 rpm for 3 min and the absorbance of the supernatant was measured at 540 nm using spectro-photometer [30]. The percentage inhibition of hemolysis was calculated thus;

Inhibition of hemolysis (%) = [1- (OD2-OD1/OD3-OD1)] x 100

OD1 = absorbance of test sample in isotonic solution
OD2 = absorbance of test sample in hypotonic solution
OD3 = absorbance of control sample in hypotonic solution

### 2.8 Statistical Analysis

The results were presented as mean + SEM. The statistical analyses were performed using ANOVA (SPSS 11.5). Dunnett's test was performed as post-hock test. Differences between means of groups were considered significant at $p < 0.05$.

## 3. RESULTS

### 3.1 Acute Toxicity and Phytochemical Analysis

Oral administration of the ethanol extract up to 5000 mg/kg did not produce obvious signs of toxicity or mortality in rats. The $LD_{50}$ of the extract is thus greater than 5000 mg/kg. The ethanol extract had high amount of saponins and tannins and moderate amount of alkaloids, flavonoids, reducing sugars, steroids and cardiac glycosides. The phytoconstituents present in the ethylacetate fraction were mainly flavonoids and tannins (Table 1).

### 3.2 Effect of EE and ETF on Egg Albumin Induced Acute Inflammation

Sub-planta injection of egg albumin evoked a progressive increase in the rat paw edema. Both the extract and ethylacetate fraction caused a progressive and significant ($p < 0.05$) inhibition of edema which peaked by the 4$^{th}$ hour (Table 2). The percent edema inhibition by at the 4$^{th}$ hour by EE was 16.07 and 19.64 for 400 and 600 mg/kg respectively, while that of ETF at the same time and corresponding doses was 50.88 and 49.1 respectively. The inhibition by ETF was more than that of aspirin (43.5%) (Figure 1)

**Table 1. Phytochemical constitute of the extract and fraction**

| Phyto -compounds | Ethanol Extract | n- hexane fraction | Ethylacetate fraction | Butanol fraction | Water fraction |
|---|---|---|---|---|---|
| Alkaloid | ++ | - | - | + | - |
| Saponin | +++ | - | - | ++ | + |
| Flavonoid | ++ | - | ++ | + | - |
| Tannin | +++ | - | +++ | + | - |
| Reducing sugar | ++ | - | - | ++ | - |
| Anthraquinone | - | - | - | - | - |
| Steroid | ++ | ++ | - | - | - |
| Protein | + | - | + | - | - |
| Cardiac glyiosides | ++ | - | - | + | ++ |

*Key + = present, - = Absent, ++ moderately abundant*
*+++ = Abundant*

**Table 2. Effect of the extract and ethylacetate fraction on egg albumin-induced rat paw edema**

| Fractions | Dose (mg/kg) | Mean edema (ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| EE | 200 | 0.48±0.07 | 1.08±0.05 | 0.968±0.07 | 0.98±0.06 | 0.84±0.05 | 0.80±0.05 | 0.70±0.06 |
| | 400 | 0.50±0.05 | 1.05±0.04 | 0.96±0.07 | 0.94±0.08* | 0.55±0.06* | 0.54±0.04* | 0.55±0.04* |
| | 600 | 0.50±0.06 | 1.00±0.06 | 0.95±0.05 | 0.90±0.06* | 0.54±0.05* | 0.54±0.06* | 0.54±0.05* |
| ETF | 200 | 0.60±0.06 | 1.14±0.08 | 1.10±0.06 | 0.96±0.06 | 0.60±0.06* | 0.60±0.06* | 0.60±0.06* |
| | 400 | 0.55±0.05 | 1.14±0.06 | 1.05±0.05 | 0.84±0.06 | 0.56±0.06* | 0.56±0.04* | 0.56±0.05* |
| | 600 | 0.56±0.07 | 1.12±0.06 | 0.90±0.04 | 0.74±0.05* | 0.55±0.05* | 0.55±0.05* | 0.55±0.06* |
| Aspirin | 100 | 0.55±0.05 | 1.14±0.05 | 1.00±0.05 | 0.82±0.04* | 0.65±0.06* | 0.62±0.06* | 0.60±0.06* |
| 10% Tween 80 | 10 ml/kg | 0.55±0.06 | 1.20±0.07 | 1.18±0.06 | 1.16±0.07 | 1.15±0.06 | 1.05±0.08 | 0.90±0.06 |

** p < 0.05, EE=Ethanol extract, ETF=Ethylacetate fraction*

**Table 3. Effect of extract and fraction on xylene induced topical edema of the mouce ear**

| Treatment | Dose (ug/ear) | Mean left ear (mg) | Mean right ear (mg) | Percentage inhibition (%) |
|---|---|---|---|---|
| Ethanol extract | 200 | 4.9±0.07 | 9.2±0.07* | 33.85 |
| | 400 | 4.1±0.06 | 8.0±0.08* | 40.00 |
| | 600 | 4.33±0.05 | 8.0±0.06* | 43.08 |
| E. acetate fraction | 200 | 4.8±0.03 | 8.8±0.04* | 38.46 |
| | 400 | 5.5±0.06 | 8.4±0.06* | 55.38 |
| | 600 | 6.3±0.04 | 8.6±0.05* | 64.62 |
| Indomethacin | 200 | 4.6±0.08 | 8.2±0.06* | 44.62 |
| Tween 80 (µl) | 50 | 4.6±0.05 | 11.1±0.08 | - |

*n = 6, * p < 0.05*

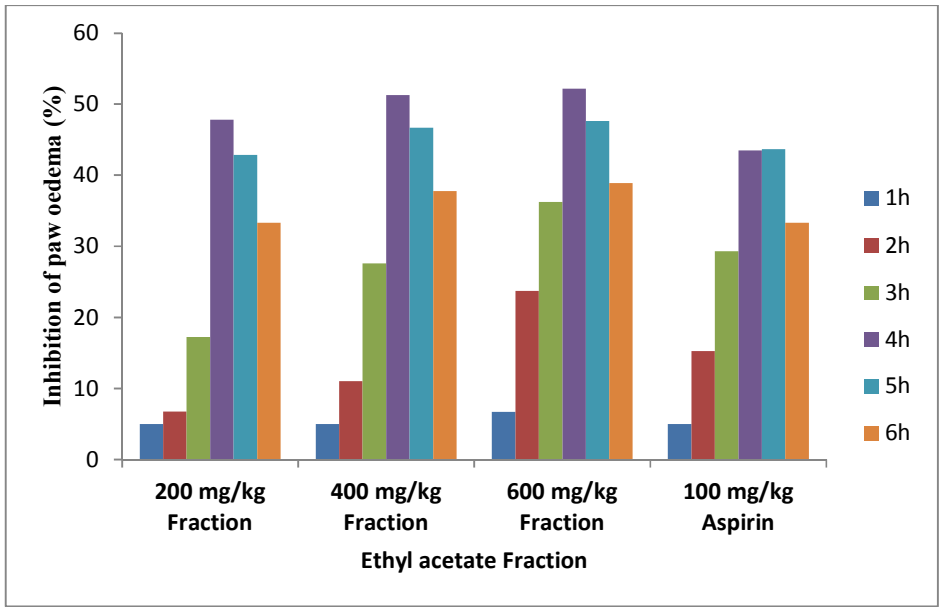


**Figure. 1. Inhibitory effect of the ethylacetate fraction on egg albumin-induced rat paw edema**

### 3.3 Effect on Xylene-induced Edema

Topical application of the EE and ETF significantly ($p < 0.05$) reduced xylene-induced inflammation in a dose related manner. The percent edema inhibition by ETF at all the doses were greater than that of EE at equivalent doses and also greater than that of indomethacin (Table 3)

### 3.4 Ulcerogenic Effects in Rats

The extract and ethylacetate fraction produced minimal deleterious effects on the integrity of gastric mucosa compared to indomethacin. The ulcer index of EE and ETF-treated rats (1.2-1.8) were significantly ($p < 0.05$) less than that of indomethacin treated group (5.2) (Table 4)

### 3.5 Effect of the Extract and Ethylacetate Fraction on *In vivo* Leucocytes Migration in Rats

Pre-treatment with the EE and ETF significantly ($p < 0.05$) reduced agar-induced leucocyte migration. At 200, 400 and 600 mg/kg the percent inhibition by EE was 15.40, 26.73 and 38.39 respectively, and for ETF, 29.71, 42.21 and 58.79 respectively. The inhibition by ETF (600 mg/kg, 58.79%) was high than that of indomethacin (50.42%) (Table 5). The neutrophils were the most suppressed leukocytes.

**Table 4. Ulcerogenic effect of extract and ethylacetate fraction**

| Treatment | Dose (mg/kg) | Ulcer index (Mean ±SEM) |
|---|---|---|
| Ethanol Extract | 200 | 1.20±0.05 |
| | 400 | 1.20±0.02 |
| | 600 | 1.20±0.04 |
| Ethyl acetate fraction | 200 | 1.50±0.06 |
| | 400 | 1.50±0.10 |
| | 600 | 1.60±0.06 |
| Indomethacin | 40 | 5.20±0.23* |
| Control 10% Tween 80 | 10ml/kg | 0.65±0.04 |

*n= 6, *p < 0.05*

### 3.6 Membrane Stabilizing Effect

Both EE and ETF significantly ($p < 0.05$) inhibited heat-induced heamolysis of human RBC membrane, while hypotonicity-induced haemolysis was not significantly affected (Tables 6 and 7)

## 4. DISCUSSION

The very high $LD_{50}$ of the extract (> 5000 mg/kg) in addition to its lack of obvious toxicity indicate that the EE and ETF will be relatively safe in the management of chronic inflammatory disorders such as rheumatoid arthritis. Earlier study on the cytotoxicity of the extract of this plant confirmed that it did not induce cell death [19].

**Table 5. Effect of the extract and ethylacetate fraction on *in vivo* leucocytes migration**

| Treatment | Dose mg/kg | TLC ($mm^3$) | % inhibition | Differential Leucocyte count ($mm^3$) | | |
|---|---|---|---|---|---|---|
| | | | | N | M | L |
| Ethanol | 200 | 3235±365 | 15.40 | 13.32 | 5.68 | 81.00 |
| Extract | 400 | 2802±256* | 26.73 | 11.85 | 7.10 | 81.05 |
| | 600 | 2356±129* | 38.39 | 9.95 | 9.70 | 80.35 |
| Ethyl acetate | 200 | 2688±143* | 29.71 | 12.90 | 6.60 | 80.50 |
| Fraction | 400 | 2210±222* | 42.21 | 9.20 | 10.45 | 80.35 |
| | 600 | 1648±198* | 58.79 | 3.56 | 13.80 | 82.64 |
| Indomethacin | 50 | 1896±321* | 50.42 | 4.80 | 15.60 | 79.60 |
| 10% Tween 80 | 10 ml/kg | 3824±288 | - | 13.05 | 10.50 | 76.45 |

*n= 6, * p < 0.05 where N = Neutrophils, M = Monocytes, L = Lymphocytes*

**Table 6. Effect of extract and ethylacetate fraction on heat induced haemolysis of human red blood cell membrane**

| Treatment | Conc ( µg/ml) | Absorbance Refrigerated samples | Absorbance heated samples | % inhibition |
|---|---|---|---|---|
| Ethanol extract | 200 | 0.62±0.002 | 0.91±0.002 | 40.82 |
| | 400 | 0.60±0.004 | 0.87±0.007* | 46.08 |
| | 600 | 0.58±0.002 | 0.77±0.004* | 64.10 |
| E acetate fraction | 200 | 0.54±0.001 | 0.82±0.001* | 50.13 |
| | 400 | 0.52±0.002 | 0.66±0.002* | 76.35 |
| | 600 | 0.55±0.002 | 0.66±0.003* | 80.43 |
| Prednisolone | 200 | 0.53±0.001 | 0.64±0.002* | 80.10 |
| Control | 10% Tween 80 | 0.83±0.002 | 1.11±0.005 | - |

** p < 0.05*

**Table 7. Effect of the extract and ethylacetate fraction on hypotonicity induced haemolysis of human red blood cell membrane**

| Treatment | Conc (µg/ml) | Absorbnce Isotonic solution | Absorbance Hypotonic solution | % inhibition |
|---|---|---|---|---|
| Ethanol extract | 200 | 0.44±0.007 | 0.91±0.006 | 9.62 |
| | 400 | 0.40±0.005 | 0.85±0.005* | 19.65 |
| | 600 | 0.37±0.003 | 0.57±0.005* | 66.63 |
| Ethyl acetate fraction | 200 | 0.40±0.002 | 0.88±0.002* | 14.34 |
| | 400 | 0.35±0.006 | 0.58±0.002* | 59.00 |
| | 600 | 0.32±0.001 | 0.50±0.005* | 71.90 |
| Indomethacin | 200 | 0.32±0.003 | 0.48±0.003* | 75.00 |
| Control | | 0.48±0.002 | 0.96±0.002 | |

** p < 0.05*

Anti-inflammatory properties of some medicinal plants have been attributed to their saponins [31] and steroidal [32] contents. The anti-inflammatory activities of flavonoids and tannins have been linked to their antioxidant properties [33,34]. The ethylacetate mainly extracted these two phytoconstituents, and the copious presence of these two metabolites may explain the potent and stronger anti-inflammatory activity exhibited by ETF.

Release of histamine and serotonin have been associated with early inflammatory response lasting up to 2 hr post administration of the irritant while the later phase, occurring from 3 – 5 hr is associated with bradykinins, proteases, prostaglandins, lysosomes and oxygen derived free radicals [35,36]. The significant reduction in paw edema by EE and ETF from the 3$^{th}$ hr could result from the inhibition of the release or antagonism of the actions of later phase inflammatory mediators. Flavonoids have been reported to down-regulate inflammatory mediators and to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation [37,38]. The

extract of *E. indica* have been reported to possess a free radical scavenging activity [39], which could play a beneficial role against pathological alterations like edematous conditions.

Xylene is a phlogistic agent that act on target cells in the periphery like mast cells, immune cells, and vascular smooth muscle, and promote neurogenic inflammation [40]. Flavanones have been documented to ameliorate ear edema induced by xylene [40]. The ability of flavonoids to inhibit NADH oxidase system in mast cells is believed to play a role in their anti-inflammatory properties [41]. The presence of flavonoids in the extract and its abundance in the ethylacetate fraction may have contributed to the topical effect recorded in this study.

Anti-inflammatory agents that inhibit prostaglandin synthesis are prone to cause irritation or ulceration of the gastrointestinal tract. Also generation of reactive oxygen species as occurs with some NSAIDs can result in gastrointestinal track injury. The extract and ethylacetate fraction produced minimal deleterious effects on the integrity of gastric mucosa. The plant could thus be beneficial for inflammatory pains and swelling as seen in rheumatoid arthritis with reduced risk of gastric ulcer.

Pre-treatment with the extract and ethylacetate fraction significantly ($p < 0.05$) reduced leucocyte migration. The later phase of acute inflammatory response involves cellular migration to the site of inflammatory stimulus [42]. Inhibition of leucocyte migration by the extract and fraction is suggestive of effect in the later phase of inflammation. Neutrophils which engulf and eliminate microorganisms were the most suppressed leukocytes. They are the first line of defence in the immunological response against pathogens. In inflammatory conditions, they present a potential risk of tissue damage [43], by their interaction with local inflammatory mediators which leads to production of several other mediators that amplify the inflammatory response and tissue damage [44]. These results suggest that the anti-inflammatory effect of the extract and fraction may result from the alteration of inflammatory responses via modulation of cellular and molecular mediators involved in inflammatory pathways.

The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupture and busting of its membrane while heat leads to shrinkage and oxidative busting of the cell [45]. Leakage of serum proteins and fluids into the tissue during a period of inflammation-induced increased vascular permeability is reduced by membrane stabilization [46]. Though the precise mechanism of this membrane stabilization has not been ascertained, it is possible that increase in surface area/volume ratio of the cells as a result of membrane expansion or shrinkage of the cell, and an interaction with cell membrane proteins may have occurred. It is also possible that the membrane stabilization effect of the extract and fraction may be traced to their ability to alter the influx of calcium into the erythrocytes [30]. In addition to inhibition of leucocytes migration, the extract and the ethylacetate fraction also may prevent the release of cytoplasmic pro-inflammatory mediators through membrane stabilization.

## 5. CONCLUSION

These results revealed that the leaves of *Eleusine indicia* are endowed with potent anti-inflammatory activity. The anti-inflammatory activity resides mainly in the ethylacetate fraction. The extract and ethylacetate fraction lack gastrointestinal irritation and ulceration, a limitation in the use of NSAIDs. Inhibition of leucocytes migration and membrane stabilization appear to mediate the anti-inflammatory activity of the leaves of *Eleusine indica.*

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All animal experiments were conducted in compliance with the NIH guide for care and use of laboratory animals (Pub. No. 85-23 revised 1985), and approved by the Ethical Committee on the use of Laboratory Animal of Nnamdi Azikiwe University, Awka, Nigeria.

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## COMPETING INTERESTS

Authors have declared that no competing interest exists.

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