Trends in
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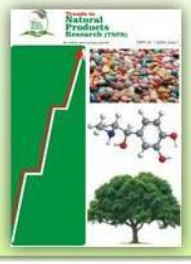

Evaluation of the Anti-nociceptive Effect of the Ethanol Leaf Extract and Ethyl Acetate Fraction of *Eleusine indica* Linn, Baertn.

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Key words: *Eleusine indica*, Anti-nociceptive, Ethyl Acetate, Phytoconstituents.

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Abstract Efforts made over the decades to develop effective therapies for pain have been hampered by the cost and adverse effects of conventional anti-nociceptive. There is still need for effective, potent and safe drugs for pain. This study investigated the anti-nociceptive activity of the ethyl acetate fraction of the ethanol leaf extract of *E. indica* in rodents. The leaves were extracted with ethanol by cold maceration and the extract was subjected to bioassay-guided liquid-liquid chromatographic separation to obtain n-hexane, ethyl acetate, butanol and aqueous fractions. Only the ethylacetate fraction (ETF) exhibited significant anti-nociceptive activity. The oral acute toxicity (LD_{50}) of the extract and the phytochemical constituents of the extract and the ethyl acetate fraction were determined. Pain was induced in mice experimentally using hot plate and acetic acid. The experimental mice were given 200, 400 and 600 mg/g of the extract or fraction. Tween 80 (3 % w/v), morphine or piroxican served as control treatments. The oral LD_{50} of the extract was above 5000 mg/kg. Saponins, tannins, flavonoids, alkaloids and steroids were the most abundant phytocompounds in the EE while ETF contained mainly tannins and flavonoids. Against thermal induced nociception the extract and fraction evoked significant ($p < 0.05$) prolongation of the reaction time which peaked 90 minutes post administration. At dose of 600 mg/kg of both the extract and fraction, and 50 mg/kg of diclofenac sodium, the post treatment reaction time at 90 min was 8.8±0.3, 10.5±0.5 and 15.8±0.6 seconds, respectively. Both the EE and ETF displayed dose-dependent and significant ($p < 0.05$) inhibition of acetic acid-induced writhing. At 600 mg/kg, the ethyl acetate fraction produced 67.71 % inhibition of

writhing reflex that was similar to 65.92 % produced by piroxicam (100 mg/kg). The findings of this study indicate that the leaves of *Eleusine indica* have potential in the treatment of pain and that the anti-nociceptive activity may be attributed to phytoconstituents present mainly in the ethyl acetate fraction.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Craig, 2006). Pain is an unpleasant perception and a nociceptive sensation (Kumar and Elavarasi, 2016), that arises from a complex interaction between nociceptive stimulus and different emotional and cognitive factors, which appear to be mediated by both automatic and controlled systems (Pelaez *et al.*., 2016). Pain is a complicated experience integrating prior exposures, expectations, attention, mood, genetics, peripheral and central nervous system physiology as well as neurochemical and anatomical variation (Oudejans, 2016). Pain can originate from any situation; injury being the major cause. Pain may be an indicator of tissue damage but may also be experienced in the absence of an identifiable cause. The degree of disability experienced in relation to the experience of pain varies; similarly there is individual variation in response to methods pain alleviation (Eccleston, 2001). Pain is more than a sensation or the physical awareness of pain; it also includes perception, the subjective interpretation of the discomfort.

Pain may be acute or chronic. Acute pain is of recent onset and probable of limited duration. It usually has an identifiable temporal and causal relationship to injury or disease (Woolf and Ma, 2007). Acute pain is provoked by identifiable stimuli and disappears as soon as the tissue injury or primary damage that had caused it is abated (Malek and Bejsovec, 2017). Chronic pain persists beyond the time of healing of an injury and in most cases there may not be any clearly identifiable cause (Quinter *et al.,* 2008). Chronic pain is usually preceded by a focal lesion or trauma or may be a consequence of systemic diseases that disrupt peripheral small nerve fiber function and/or central modulation of nociception (Oudejans, 2016).

One of the prominent features of inflammation is that normally innocuous stimuli produce pain. (Tracey and Mantyh 2007). Pain is part of the first four classic signs of inflammation, together with blush, tumor and heat. Currently, they are recognized as elicited by a series of chemically mediated events, such as local vascular flow and patency changes, leukocytes infiltrate and algogenic substances release (Kumar and Elavarasi, 2016).After tissue injury or nerve damage, neurones along the nociceptive pathway may display enhance sensitization and responsiveness.

Medicinal plants are widely employed traditionally as analgesics and for treatment of inflammatory disorders. Herbal anti-nociceptives are readily available, affordable and arguably have fewer adverse effects. Several studies have reported the anti-nociceptive activities of medicinal plants (Akram *et al*., 2013, Islam *et al*., 2016, Regalado *et al*., 2017, Galehdar *et al*., 2018, Alatshan *et al* 2018., Rasdev *et al* 2018., Gomez-Betaneur *et al*., 2019).

In the south eastern Nigeria, a good number of plants are used traditionally to treat painful conditions. *Eleusine indica* Linn (Poaceace) commonly known as wire grass, goose grass or crowfoot grass is one of the popular traditional anti-nociceptive folk remedy. It is locally known as "Ese" in the south east, Nigeria. In this region, the leaves 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 done on the plant reported activities such as anti-diabetic (Okokon *et al*., 2010), anti-plasmodial (Okokon *et al*., 2010, Ettebong *et al*., 2012) and analgesia (Ettebong and Nwafor 2014). This study was designed to pharmacologically screen the leaves of *E. indica* for anti-nociceptive potentials.

MATERIALS AND METHODS

Materials

Chemicals, Reagents, and Drugs.

All the chemicals and reagents used for the experiments (solvents, Tween 80, 3 % w/v piroxican, morphine, acetic acid) were of analytical grade and products of Sigma Aldrich, Germany.

Animals

Swiss albino 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. All animal experiments were conducted in compliance with the NIH guide for care and use of laboratory animals (Pub. No. 85-23 revised 1985).

Methods

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 identity was established and authenticated by Mr A. Ozioko of International Centre for Drug Development (Inter-CEDD) Nsukka, Enugu State (Inter-CEDD/2014/342). The leaves were carefully separated from the woody part, cut into small pieces, sun-dried for 7 days and pulverized using a milling machine.

Extraction and Fractionation

About 1.5 kg of the pulverized 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, ethyl acetate, 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 ethyl acetate fraction (ETF) as the most effective, and were thus subjected to further studies.

Acute Toxicity (LD_{50}) and Phytochemical Studies

The oral acute toxicity (LD_{50}) of the ethanol extract (EE) was determined (Lorke, 1983), while the phytochemical analysis of both EE and the fractions were performed using standard methods (Odebiyi and Sofowora 1978, Trease and Evans 1989).

Analgesic Evaluations Hot Plate Test

The mice were fasted overnight with water allowed *ad libitum* (Imam *et al*., 2012) Oral administration of 200, 400 and 600 mg/kg of the extract or ETF were given to the groups of 6 mice each. Control groups received either morphine (5 mg/kg) or Tween 80 (10 ml/kg). The mice were individually exposed to hot plate ($55^0C \pm 1^0C$). To avoid any possible damage to the paw of the mice, 20 sec was taken as cut off period. Reaction time to the thermal stimulus noted as forepaw licking, withdrawal of the paw(s) or jumping was recorded at 10, 20, 30, 40, 50 and 60 minutes after treatment.

Acetic Acid Induced Writhing Test in Mice

This was conducted using the method of Koster *et al* (1959). Swiss albino mice of eight sex were randomly divided into eight groups (n=6). The extract or ETF (200, 400 or 600 mg/kg) were given to six groups, while the control groups received either piroxican (100 mg/kg) or Tween 80 (10 ml/kg). After 30 min of oral drug or vehicle administration, the mice were treated with 5 % acetic acid at 10 ml/kg intraperitoneally. The mice were then placed in plexi-glass for observation. The number of abdominal constrictions (writhes) was counted for each mouse for a period of 10 minutes after 5 minutes latency and the percentage inhibition of writhing calculated using the formula (Nworia *et al*., 2015);

Percentage inhibition of writhing = (Cn - Tn)/Cn x 100

Where Cn = number of writhing of vehicle group, and Tn = number of writhing of the treated groups.

Statistical Analysis

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

RESULTS

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).

Effect of the Extract and Fraction on Hot Plate-induced Pain

The ethanol extract (600 mg/kg) and ETF (400 mg/kg) significantly increased the latency to thermal nociception by the 90^{th} min ($p < 0.05$). The ETF (600 mg/kg) evoked significant ($p < 0.05$) increase latency to the thermal stimulus from 60^{th} min (Figure 1). Peak effects of the extract and fraction occurred 90 minutes post administration.

Effect of the Extract and Fraction on Acetic Acid-induced Pain

Administration of the extract and fraction generally and significantly ($p < 0.05$) reduced acetic acid-induced pain (Figure 2). This was evident from the percentage reduction in number of abdominal constrictions. The extract produced significant ($p < 0.05$) inhibition of writhing reflex at 400 and 600 mg/kg while the inhibition by ethyl acetate fraction was from 200 mg/kg. The inhibition by ethyl acetate fraction (67.71 %) at 600 mg/kg was similar to that of piroxicam (65.71 %) (Figure 3).

Table 1. Phytochemical Constituents of the Extract and Fraction

Phyto -compounds	Ethanol Extract	n- hexane fraction	Ethylacetate fraction	Butanol fraction	Water fraction
Alkaloids	++	-	-	+	-
Saponins	+++	-	-	++	+
Flavonoids	++	-	++	+	-
Tannins	+++	-	+++	+	-
Reducing sugars	++	-	-	++	-
Anthraquinones	-	-	-	-	-
Steroids	++	++	-	-	-
Protein	+	-	+	-	-
Cardiac glyiosides	++	-	-	+	++

Key + = present, - = absent, ++ moderately abundant +++ = Abundant

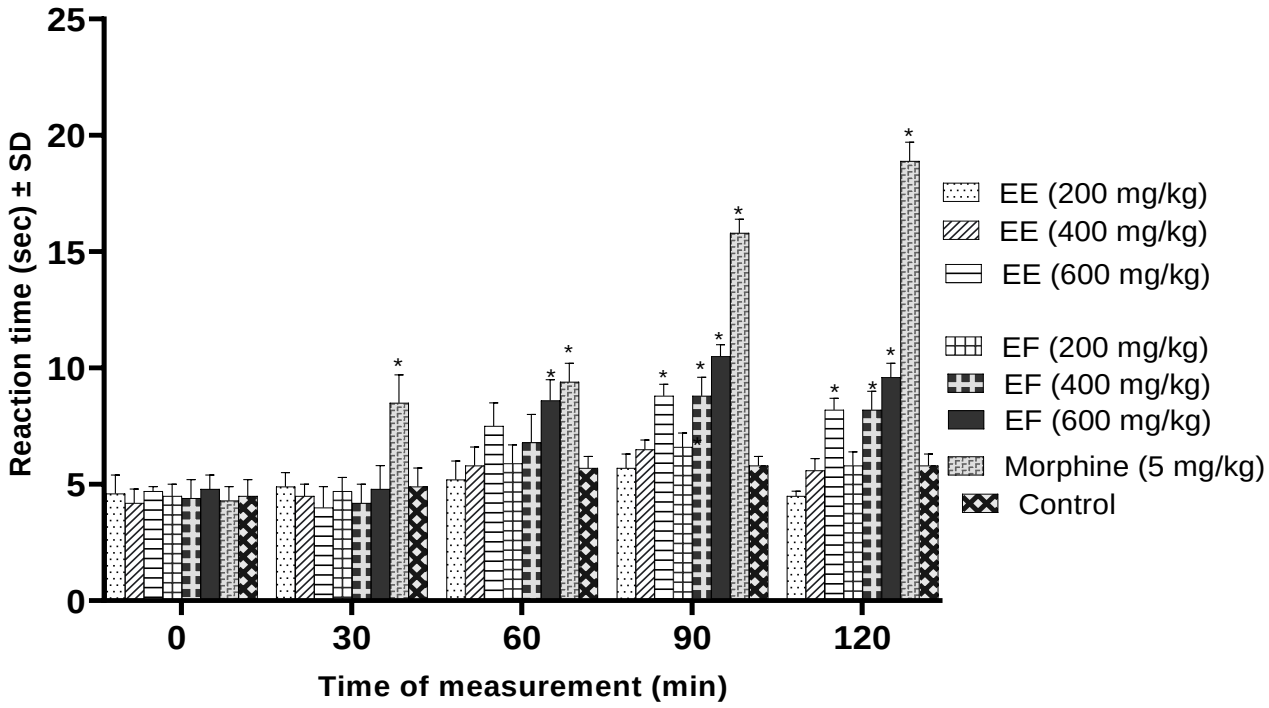

Figure 1. Analgesic Effect of Extract and Fraction by Hot Plate Method. *$p < 0.05$, n=6

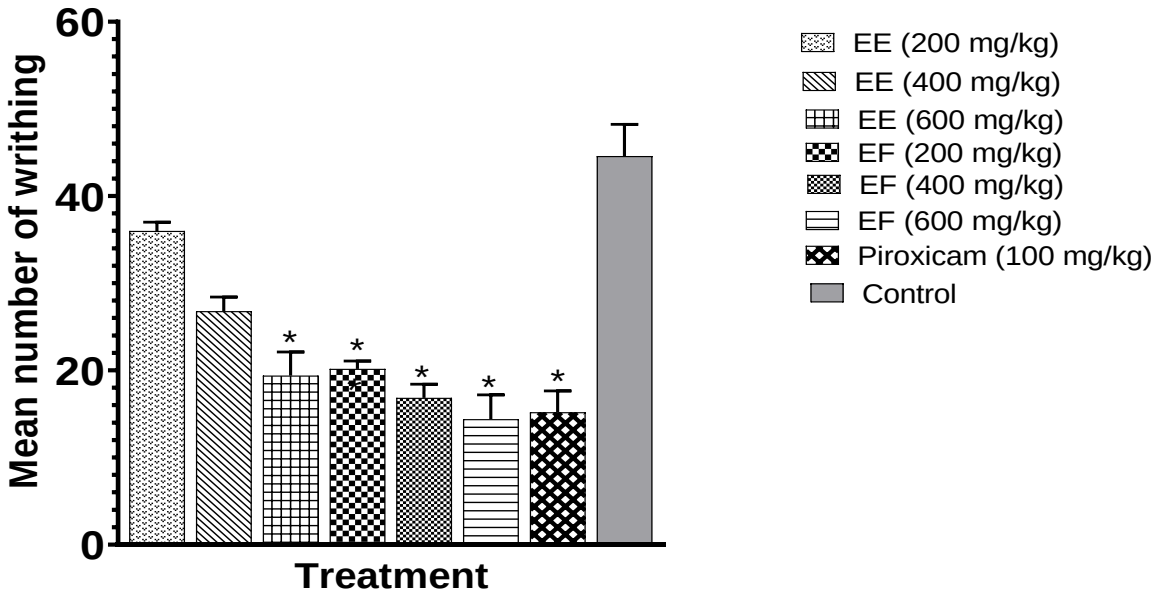

Figure 2. Effect of Extract and Fraction on Acetic Acid Induced Writhing Reflex. *$p < 0.05$, n=6

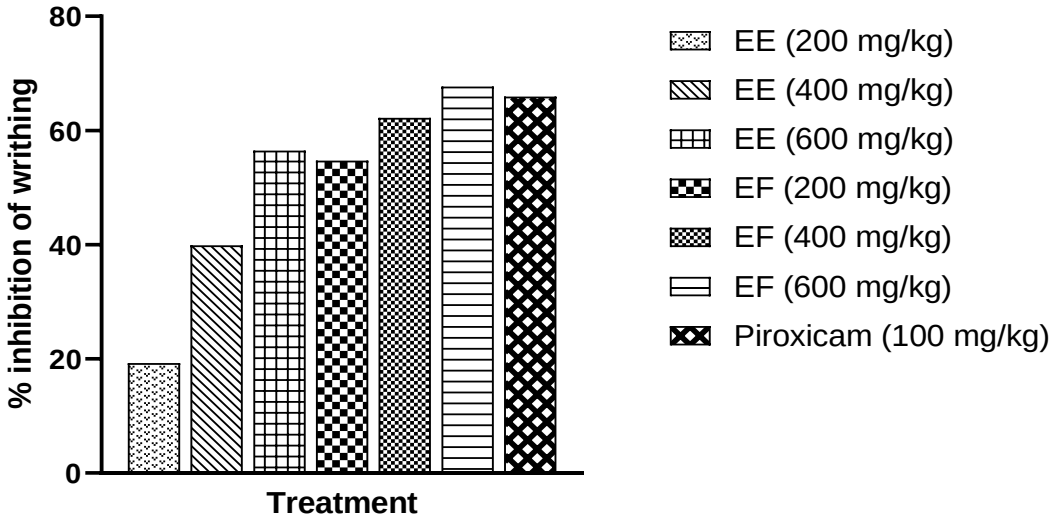

Figure 3. Percentage Inhibition of Acetic Acid-Induced Abdominal Writhes by the Extract and Fraction

DISCUSSION

The results of this study indicate that the leaves of *E. indica* possess significant antinociceptive activity. This activity apparently resides more in the ethyl acetate fraction of the ethanol extract of the leaves. Preliminary qualitative phytochemical screening revealed the presence of bioactive constituents endowed with pain relieving properties. Hot plate model is believed to demonstrate the supraspinal reflex mediated via $\mu_1$and μ_2 opoid receptors (Arslam and Bektas, 2010) and is therefore of central origin. This method is therefore useful in screening compounds effective in spinal and supraspinal region. Most therapeutic agents effective in modulation of this pain response act supraspinally rather than peripherally (Gholami *et al.*, 2015). The increase in latency in this pain model by the extract and fraction suggests the modulation of central nervous system pain pathway (Nasrin *et al.*, 2017). The involvement of central opiod receptors in the antinociceptive effect of plants extract have been documented (Nasrin *et al*., 2017). Some centrally acting antinociceptive agents are postulated to inhibit the nociceptive effect of 5-hydroxytryptamine, adrenaline, noradrenaline, prostaglandins, bradykinins, acetylcholine and adenosine (Gholami *et al.*, 2015). Similar mechanism of action has been postulated for *Cyperus rotundus* (Imam and Sumi 2014). The extract and ethyl acetate fraction may have acted through any of these pathways.

Acetic acid-induced abdominal constriction is a sensitive method widely accepted in screening peripheral analgesic effect (Adeyemi *et al.*, 2011), and it is believed to involve local peritoneal receptors (Bentley *et al*., 1983). Acetic acid is known to enhance the synthesis of prostaglandins through the release of arachidonic acid (Davies *et al*., 1984), thus acetic acid-induced abdominal analgesia is due to peripheral sensitization by prostaglandins. It therefore follows that agents that inhibit acetic acid-induced writhing could be effective in ameliorating peripheral pain through inhibition of prostaglandin production The extract and fraction were more effective in inhibiting acetic acid-induced pain than thermal pain. Their mechanism of action can be postulated to be similar to that of non-steroidal anti-inflammatory drugs like piroxicam which relieve pain by inhibiting prostaglandin production by blocking cyclooxygenase (COX1) pathway. Release of pain mediators such as histamine, prostaglandins, bradykinins and serotonin has been associated with acetic acid (Ikedia *et al*., 2001). Inhibition of the release or/and antagonism of the actions of these mediators by the extract and fraction may contribute to their potent inhibition of acetic acid-induced nociception and by extension their antinociceptive effect. The phytoconstituents detected in the extract and fraction may account for their antinociceptive effect. Flavonoids (Miladi-Gorgi *et al*., 2005, Manjunatha *et al*., 2006), alkaloids (Mei *et al*., 2011) and terpenoids (Juma *et al*., 2015) have been reported to demonstrate antinociceptive activities.

CONCLUSION

These results strongly support medicinal plants with bioactive compounds to provide an excellent avenue

to alleviate pain. The ethanol leaf extract of *Eleusine indica* and its ethyl acetate fraction demonstrated potent antinociceptive effect against thermal and chemical-induced pain, with the ethyl acetate fraction exhibiting more antinociceptive effect. The extract and fraction exhibited both peripheral and central antinociceptive potentials.

CONFLICT OF INTEREST

This study did not receive any specific grant from funding agencies in the public, commercial or non-profit sector. The authors declare no conflicting interest

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