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Research Article

Evaluation of analgesic and behavioural effects of ethanol root bark extract of *Erythrina senegalensis* DC (Fabaceae)

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Background: The ethnomedicinal uses of *Erythrina senegalensis* including its antinociceptive and sedative properties have been documented in literature.

Objective: This study evaluated the analgesic and behavioural effects of the ethanol root bark extract of *E. senegalensis* in mice.

Methodology: Phytochemical screening and acute toxicity studies were conducted. Analgesic activity in mice was assessed using acetic acid induced writhing and hot plate method, while behavioural effects were evaluated using diazepam-induced sleeping test and hole-board test. These evaluations were carried out on *E. senegalensis* ethanol root bark extract at doses of 75, 150 and 300 mg/kg.

Results: The intraperitoneal median lethal dose was found to be 1,137 mg/kg, while alkaloids, flavonoids, saponins, tannins and reducing sugars were found to be present in the plant material. *E. senegalensis* ethanol root bark extract at 150 and 300 mg/kg exhibited significant ($p < 0.001$) analgesic activity which offered 17.6% and 25.8% inhibition above ketoprofen in the acetic acid test respectively. At 300 mg/kg, *E. senegalensis* ethanol root bark extract demonstrated comparative analgesia with pentazocine in hot plate test. At the same dose, it produced a significant ($p < 0.05$) potentiation of diazepam-induced sleeping time. A significant increase in number of head-dips was demonstrated by *E. senegalensis* ethanol root bark extract at 150 mg/kg.

Conclusion: The study shows that *E. senegalensis* ethanol root bark extract possesses analgesic, sedative and anxiolytic principles, thus supporting the ethnomedicinal rationale for its uses in management of painful conditions and sleep disturbances.

Keywords: *Erythrina senegalensis*, analgesic, sedative, behavioural

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1. Introduction

Traditional herbal medicines are naturally occurring plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices (Tilburt and Kaptchuk, 2008). The number of patients seeking alternate and herbal therapy is growing exponentially as herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects (Biren and Avinash, 2010; WHO, 2013). Herbal remedies are utilized for many purposes, amongst which several species of plants have been studied for their role in management of pain and central nervous system (CNS) related problems. One of these species is *Erythrina senegalensis*.

Erythrina is a large genus of flowering plants in the pea family also known as Fabaceae. It consists of over 100 species of plants accepted and widely distributed in

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tropical and subtropical regions of the world (Enusic et al, 2002, Gledhill, 2008).The plant *Erythrina senegalensis* DC is a thorny shrub or small tree commonly known as coral tree (English) and *Minjirya* (Hausa, Nigeria). Different parts of *E. senegalensis* (leaves, stem and root barks) are used by traditional healers to cure wide range of illnesses (Togola et al, 2008; Kone et al, 2011).The leaves are used to treat malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice and pain, while the roots are used in the treatment of neuralgic malaria, insomnia, dysmenorrhoea, pneumonia and nose bleeding (Togola et al, 2008).

Various scientific studies had established the hepatoprotective, antimicrobial and antiviral activities of the stem bark of *E. senegalensis* (Donfack et al, 2008; Lee et al, 2009; Doughari, 2010). The stem bark had also been shown to possess positive chronotropic effect (Nembo et al, 2015), cytotoxic activity (Kuete et al, 2014), analgesic and anti-inflammatory properties (Saidu et al, 2000; Vasconcelos et al, 2003). The root extract exhibited strong activity against *Plasmodium falciparum* (Atindehou et al, 2004). Despite the wide use of the root parts of *Erythrina senegalensis* in ethnomedicine for the management of pains and insomnia, there is no reported scientific evidence to validate its efficacy. Therefore, the current study was aimed at evaluating the ethanol root bark extract of *Erythrina senegalensis* for analgesic and sedative potentials in animal models not only to validate its usefulness in ethnomedicine but as a preliminary step towards development of a more efficacious plant-derived agents with analgesic and sedative properties.

2. Materials and Methods

2.1 Drugs and chemicals

Diazepam (Roche, France), Ketoprofen (M&B) and Pentazocine (Ranbaxy), Acetic acid (BDH) and Ethanol (Sigma Aldrich, USA) were used. All dilutions and drug preparation were done using distilled water. The extract was also freshly prepared using distilled water before each experiment.

2.2 Animals

Swiss albino mice of either sex weighing 18-22 g were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained in well ventilated room (at room temperature) and fed on standard animal feed (Feeds Master, Ilorin, Nigeria) and water *ad libitum*. The experimental protocols were approved by the Ahmadu Bello University Animal Ethics Committee.

2.3 Plant material

Erythrina senegalensis roots were collected in the month of October, 2014 from Tudun-Wada Dankade Local Government Area of Kano State, Nigeria. The plant specimen was identified and authenticated by a taxonomist in the herbarium section of Department of Plant Biology, Bayero University, Kano, and a voucher specimen number (**BUKHAN 0310**) was deposited in the herbarium for future reference.

2.4 Preparation of plant extract

The root bark was cleaned, and the bark was carefully removed and air-dried to a constant weight. It was then pulverized into coarse powder using a mortar and pestle. About 100 g of the powdered root bark was extracted by cold maceration with 2.5 L of 70%$^{v}/_{v}$ aqueous ethanol with constant shaking for 3 days and then filtered using Whatman filter paper No 1.The filtrate was then concentrated to dryness over a water bath at about40ºC and the yield was calculated.

2.5 Phytochemical screening

The phytochemical constituents of the plant material were determined using standard methods as described by Evans, 2002. The crude ethanol root bark extract was subjected to phytochemical analysis for classes of chemical constituents including alkaloids, anthraquinones, flavonoids, reducing sugars, saponins, steroids and tannins.

2.6 Acute toxicity studies

Acute toxicity studies was carried out on mice of either sex using the method described by Lorke, 1983.The study was carried out in two phases; in phase 1, three groups of three mice per group were used. The first, second and third groups of mice received the extract at a doses of 10, 100 and 1000 mg/kg body weight respectively through the intraperitoneal route (*i.p.*), and the animals were observed for signs of toxicity and death within 24 hrs. In phase 2, three groups each consisting of 1 mouse were treated with 1600, 2900 and 5000 mg/kg of the extract, *i.p.*, (based on the results of first phase) and also monitored for signs of toxicity and death within 24 hrs. The median lethal dose (LD_{50}) was estimated by calculating the geometric mean of the lowest lethal dose and the highest non-lethal dose.

2.7 Analgesic studies

Acetic acid induced-writhing test in mice

The method of Koster et al, (1959) was adopted in this study. Five groups of six mice each were used. The first group received distilled water (10 ml/kg, *i.p.*) and served as negative control while the second, third and fourth groups received *E. senegalensis* extract at doses 75, 150 and 300 mg/kg *i.p.* respectively. The fifth group received Ketoprofen (10 mg/kg, *i.p.*) which served as standard. After thirty minutes, each mouse was injected with 10 ml/kg of 0.6%$^{v}/_{v}$ aqueous solution of acetic acid *i.p.* Five minutes after acetic acid injection, the mice were placed in individual observation cages and the number of abdominal constriction/writhes was recorded for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes using the formula:

$$\% \text{ Inhibition} = \frac{\text{Mean no. writhes (control)} - \text{Mean no. writhes (test)}}{\text{Mean no. writhes (control)}} \times 100$$

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Hot plate test in mice

This method was carried out as described by Eddyand Leimbach (1953).The temperature of the thermostat hot plate was set at 55±1°C (Mishra et al, 2011). Thirty Swiss albino mice of both sexes were used for the experiment and were divided into five groups of six mice each. The first group received distilled water (10 ml/kg *i.p.*) and served as negative control whiles the second, third and fourth groups received the extract at doses 75, 150 and 300 mg/kg *i.p.* respectively. The fifth group was set as standard and received pentazocine 20 mg/kg, *i.p*. The mice were individually placed on the thermostat hot plate in order to obtain its response to nociceptive pain stimulus. Licking of the paws or jumping off the plate was an indicator of the animal's response to heat-induced nociceptive pain stimulus. The latency for each mouse to either lick or jump off the hot plate was recorded (reaction time) using a stop-watch before and after 30, 60, 90 and 120 minutes of treatment.

2.8 Behavioural studies

Diazepam-induced sleeping test in mice

The method described by Rakotonirina et al, (2001) was adopted to study the sleep potentiating effect of *E. senegalensis* extract. The test was carried out on four groups of six mice each. The first group (control) was administered distilled water (10 ml/kg) *i.p.*, while the second, third and fourth groups received the extract at doses of 75, 150 and 300 mg/kg (*i.p.*) respectively. Thirty minutes post treatment; all the animals received diazepam 20 mg/kg body weight *i.p*. The criteria for sleep was considered to be loss of righting reflex while sleeping time was measured as the time between the loss and recovery of the righting reflex.

Hole-board test for exploratory behaviour in mice

This study was conducted using a wooden apparatus measuring 40x40 cm with 16 evenly spaced holes (Perez et al, 1998). Mice were grouped into five with six mice in each group. The first group (control) received distilled water10 ml/kg *i.p.* The second, third and fourth groups were treated with the extract at doses of 75, 150 and 300 mg/kg *i.p.* respectively, while the fifth group received diazepam 0.5 mg/kg*i.p.*as standard.

Thirty minutes after treatment, the mice were placed individually on the wooden board and allowed to explore for a period of 5 minutes. The number of head-dips into the holes made by each mouse during the five min. was recorded.

2.9 Statistical analysis

Results were presented as Mean ± Stand Error of the Mean (S.E.M.) and as percentages where appropriate. Data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Repeated measured ANOVA and Bonferroni post hoc test were used to analyze the mean reaction time in hot plate test. Values of $p \leq 0.05$ were considered significant.

3. Results

Percentage yield of ethanol root bark extract of *E. senegalensis* was 19.10%$^{w}/_{w}$. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, saponins, reducing sugars, tannins and flavonoids.

Acute toxicity studies (LD_{50} determination)

The intraperitoneal median lethal dose of ethanol root bark extract of *E. senegalensis* was estimated to be 1,137 mg/kg body weight in mice.

Effect of ethanol root bark extract of *Erythrina senegalensis* on acetic acid-induced abdominal writhing in mice

E. senegalensis root bark extract reduced the number of writhes caused by acetic acid in a dose dependent manner. The reduction was significant ($p< 0.01$, $p< 0.001$ and $p< 0.001$) at doses of 75, 150 and 300 mg/kg respectively when compared to the control group. The extract at all the doses tested also provided greater inhibition of abdominal writhes compared to the standard drug (Ketoprofen, 10 mg/kg), as shown in **Table 1**.

Effect of ethanol root bark extract of *Erythrina senegalensis* on hot plate-induced pain in mice

The ethanol root extract of *E. senegalensis* significantly ($p< 0.05$) increased the mean reaction time at different doses compared to control group (Table 3). Zero (0) min in each group was taken as control and compared to other time intervals. At 75 mg/kg of the extract, there was a significant ($p< 0.05$) increase in reaction time after 30 min, and $p< 0.01$ after 60 and 120 min. The extracts (150 and 300 mg/kg) and the standard drug (Pentazocine, 20 mg/kg) also produced a significant ($p< 0.05$) increase in reaction time at all-time intervals (**Table 2**).

Effect of ethanol root bark extract of *Erythrina senegalensis* on diazepam-induced sleeping time in mice

The ethanol root bark extract of *E. senegalensis* decreased the mean onset of sleep at all the doses tested. The decrease was significant ($p< 0.05$) at 300 mg/kg compared to the control group. The duration of sleep was also prolonged by the extract in a non-dose dependent manner which was significant ($p< 0.05$) at 300 mg/kg compared to the control group (**Table 3**).

Effect of ethanol root bark extract of *Erythrina senegalensis* on exploratory behaviour in mice

E. senegalensis root bark extract (75, 150 and 300 mg/kg) exhibited a non-dose dependent increase in the number of head dips in the hole-board experiment compared to the control group. The increase was significant ($p<0.05$) at 150 mg/kg. The standard drug, diazepam (0.5 mg/kg) also produced a significant ($p<0.05$) increase in the mean number of head dips as compared to control group (**Table 4**).

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Table 1: Effect of *Erythrina senegalensis* ethanol root bark extract on acetic acid-induced abdominal writhing in mice

Treatment	Dose (mg/kg)	Mean Number of Writhes	Percentage Inhibition (%)
Distilled water	10ml/kg	51.00±4.04	-
ERES	75	14.00±0.00**	72.5
ERES	150	11.33±3.18***	78.4
ERES	300	06.67±4.17***	86.3
Ketoprofen	10	20.30±4.91**	60.8

Values are presented as Mean **±** S.E.M., ** and *** represent p< 0.01 and p< 0.001 respectively compared to distilled water control group - One way ANOVA followed Dunnett's post hoc test, n=6,
ERES = Ethanol root bark extract of *Erythrina senegalensis*

Table 2: Effect of ethanol root bark extract of *Erythrina senegalensis* on hot plate-induced pain in mice

Treatment (mg/kg)	Mean Reaction Time (Sec.)				
	0 min	30 min	60 min	90 min	120 min
DW	1.25±0.25	2.00±0.41	2.25±0.25^{a}	2.25±0.48	1.75±0.25
ERES 75	1.75±0.25	3.00±0.00^{a}	3.25±0.25^{b}	3.25±0.25	4.75±0.25^{b*}
ERES 150	2.00±0.00	3.25±0.25^{a}	3.75±0.48^{c*}	5.00±0.71^{b*}	5.00±0.41^{b*}
ERES 300	1.25±0.25	3.75±0.25^{c*}	4.00±0.00^{c*}	5.75±0.69^{c**}	6.75±0.48^{c***}
PENTA 20	1.25±0.25	3.00±0.41^{b}	4.00±0.41^{c*}	5.25±0.48^{c*}	6.75±1.03^{c***}

Values are presented as Mean ± S.E.M., *= p<0.05, ** = p<0.01, ***= p<0.001 compared to distilled water group, while a, b, and c in superscript represent p< 0.05, p< 0.01, and p< 0.001respectively compared to time 0 (min) - repeated measure ANOVA followed by Bonferroni post hoc test; n=6,
DW = Distilled water, ERES=Ethanol root bark extract of *Erythrina senegalensis*, PENTA = Pentazocine

Table 3: Effect of ethanol root bark extract of *Erythrina senegalensis* on diazepam-induced sleeping time in mice

Treatment	Dose(mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
DW	10 ml/kg	4.25±0.25	116.25±16.66
ERES	75	3.75±0.48	164.50±37.17
ERES	150	3.50±0.29	131.50±30.44
ERES	300	3.00±0.00 $^{\#}$	251.50±18.95 $^{\#}$

Values are presented as Mean ± S.E.M., $^{\#}$ = p<0.05 as compared to distilled water control group - one way ANOVA followed by Dunnett's post hoc test, n=6,
DW = Distilled water, ERES = Ethanol root bark extract of *Erythrina senegalensis*

Table 4: Effect of ethanol root bark extract of *Erythrina senegalensis* on exploratory behaviour in mice

Treatment	Dose (mg/kg)	Number of nose poking
Distilled water	10 ml/kg	04.50±1.50
ERES	75	12.00±3.39
ERES	150	12.75±3.38*
ERES	300	11.00±1.87
Diazepam	0.5	19.75±4.10 $^{\#}$

Values are presented as Mean ± S.E.M.; $^{\#}$ = p< 0.05 compared to distilled water control group - One way ANOVA followed by Dunnett's post hoc test, n=6,
ERES = Ethanol root bark extract of *Erythrina senegalensis*

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4. Discussion

The biological actions produced by plant extracts are usually attributed to the presence of secondary metabolites in them (Kensa and Yasmin, 2011). The analgesic activity of *E. senegalensis* leaves for example have previously been attributed to the presence of phytochemicals (Saidu et al, 2000). Similarly, sedative and anxiolytic properties of some species of the genus Erythrina had been attributed to their phytochemical contents (Onusic et al, 2002). The phytochemical constituents detected in *E. senegalensis* root bark extract in this study were largely corroborative of the work of Igbokwe et al, (2006). Therefore, the analgesic and behavioural effects of *E. senegalensis* as observed in this study could possibly be attributed to its phytochemical constituents.

Using the Lorke's method of intraperitoneal acute toxicity, the LD_{50} of *E. senegalensis* root bark extract was found to be slightly toxic in mice based on the toxic classification of chemicals (Matsumura, 1975).

In the acetic acid-induced writhing test which is a common method for measuring peripheral analgesia, the significant inhibition of writhes produced by *E. senegalensis* suggests analgesic effect that involved cyclooxygenase (COX) inhibition in peripheral tissues and inhibition of prostaglandin synthesis(Vongtau et al, 2004; Odoma et al, 2014). Acetic acid-induced abdominal writhes had also been attributed to increased levels of prostanoids (prostaglandin E_2 and prostaglandin $F_{2\alpha}$) in peritoneal fluids as well as lipoxygenase products which enhance inflammatory pain by increasing capillary permeability (Voilley, 2004; Lakshman et al, 2006). Non-steroidal anti-inflammatory drugs (NSAIDs) like ketoprofen and piroxicam also reduce writhes induced by acetic acid by inhibiting COX. Thus, the analgesic activity observed with *E. senegalensis* root bark extract could be via one of the aforementioned mechanisms, which suggests possession of a peripheral analgesic activity.

The hot plate test is the most common thermal nociception model suitable for evaluation of centrally but not of peripherally acting analgesics (Vogel, 2008).In hot plate test, sensory nerves sensitize the pain receptors with minimal involvement of endogenous substances such as prostaglandins (Ezeja et al, 2011). Therefore, the effect of any drug or compound on this pain model indicates that it could have centrally acting anti-nociceptive activity (Khan et al, 2010). The significant increase in the mean reaction time produced by *E. senegalensis* root bark extract strongly suggests the presence of central anti-nociceptive effect.

Sedatives, hypnotics and neuroleptics are known to prolong diazepam induced sleeping time, while analeptics and stimulants shorten sleeping time (Vogel, 2008). *E. senegalensis* root bark extract produced a significant potentiation of diazepam induced sleep and this suggests CNS depressant property and possible sedative activity (Perez et al, 1998; Rakotonirina et al, 2001). Neurotransmitters such as dopamine, norepinephrine, acetylcholine, gamma amino butyric acid (GABA), histamine and neuropeptides (muramyl peptide) have been suggested to play important role in sleep mechanism (Guyton and Hall, 2006).

The extract increased the number of head dips in the hole-board experiment. The hole-board test is a measure of exploratory behaviour in animals (File and Wardill, 1975), and an accepted experimental model for the evaluation of psychotic, sedative and anxiety conditions in animals (Crawley, 1985). A decrease in the number of head-dips reveals a sedative behaviour and a measure of CNS depressant activity while an increase in number of head-dips reveals anxiolytic activity (File and Pellow, 1985). The increase in exploratory behaviour produced by the root extract of *E. senegalensis* reveals that it exhibited anxiolytic-like effects.

Management of pain and sedation in children is challenging, as the interplay of pain, discomfort and fear can cause agitation especially in critically ill children. According to Johnson et al, (2012), sedation and analgesia are essential to the care of critically ill children. Therefore, the combined analgesic and sedative effects observed with the root bark extract of *E. senegalensis* shows that it could stand a chance towards development of a novel compound with analgesic and sedative potentials.

5. Conclusion

The results obtained from this study showed that the ethanol root bark extract of *E. senegalensis* possess phytochemical constituent(s) with antinociceptive, sedative and anxiolytic-like activities. These findings support the ethnomedicinal claim for its efficacy in management of pain and insomnia.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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