

**EVALUATION OF ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES
OF EXTRACT OF *ERYTHROPHLEUM IVORENSE* STEM BARK IN EXPERIMENTAL
ANIMALS**

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ABSTRACT

Erythrophluem ivorense is a medicinal herb used in Africa to treat ailments such as convulsion, swellings, pain and emesis. Anti-nociceptive and anti-inflammatory activities of the crude methanol extract of *Erythrophluem ivorense* were investigated using experimental pain models, including thermal nociception like hot plate test and chemical nociception induced by intraperitoneal acetic acid or subplantar formalin injection in vivo. The anti-inflammatory property of the extract were studied using systemic administration in carrageenan-induced paw oedema. The CME administered intraperitoneally significantly ($P < 0.5$) reduced the number of abdominal constrictions by 25.8, 83.7, 88.7% at a dose of 5, 10, 20mg/kg respectively. The CME increased the reaction time on a hot plate at doses of 5, 20, 20mg/kg by 3.6 ± 0.40 , 5.8 ± 0.32 , 7.0 ± 0.50 respectively in dose related manner. CME reduced (42.5 ± 0.25 , 38.2 ± 0.50 and 35.6 ± 0.40) the paw licking time at doses of 5, 10, 20mg/kg i.p and (116.1 ± 0.05 and 67.0 ± 0.35) at doses 10 and 20mg/kg in both phases respectively. CME at doses of 5, 10, 20mg/kg inhibited paw oedema sized by 25.0, 41.7, 58.3% after application of carrageenan respectively. The study revealed that crude methanol extract (CME) of *Erythrophluem ivorense* stem bark contains components with anti-nociceptive and anti-inflammatory properties.

KEYWORDS: Erythrophleum ivorense, Anti-inflammation, Analgesic, Formalin test, Hot plate.

1.0 INTRODUCTION

Inflammation is a term derived from the latin word 'Inflammaré' which means burn. It is a complex biological response to harmful stimuli such as irritants and pathogens. It was formerly believed that inflammation is a single disease caused by disturbances in the body fluid, but according to the modern concept, inflammation is a series of chemical changes that result from the tissues injury.^[1] It is a protective process essential for maintenance of tissue homeostasis and protection against injury and infections. The sequence of events that occurs during inflammation has been grouped into three phases. These are acute transient phase, delayed sub-acute phase and chronic proliferative phase.^[2] During the acute phase, vascular permeability is enhanced and these leads to oedema around the area of inflammation. The second sub-acute phase is characterized by migration of leukocytes and phagocytes from blood to vascular tissues. This is followed by chronic phase which involves tissues degradation and fibrosis.^[3] Inflammation process also involves the

release of several pro-inflammatory mediators including cytokines (e.g tumor necrosis factor (TNF- α), interleukin (IL-1 β), chemokines, ptoteases (collagenases, elastases, metalloproteinases) and oxidants such as hydrogen peroxide and superoxide. Also, other endogenous mediators released during inflammation are histamine, prostaglandins, bradykinin and serotonin.^[4] As essential as inflammation is for the maintenance of tissue homeostasis, if left uncontrolled, it may result in several inflammatory disorders including different types of rheumatic disorders such as rheumatoid arthritis, osteoarthritis, rheumatic fever e.t.c.^[5] Inflammation is implicated in virtually all human and animal diseases, for instance, it is implicated in the pathogenesis of cancer, stroke as well as neurodegenerative diseases.^[6] Therefore, it has become a focus of global scientific research.^[7] Animal, human and in vitro models have been employed to study anti-inflammatory drugs and it has been reported that these agents produce their effects by inhibiting proinflammatory mediators.^[7] Despite the progress made in medical field during the last decades,

chronic inflammatory disorders remain one of the major health problems around the world. Most of the anti-inflammatory drugs in clinical use possess analgesic and antipyretic properties but they also cause gastric ulcer and impair blood clotting.^[8] Nonsteroidal anti-inflammatory drugs such as aspirin, ibuprofen, and naproxen are among the most widely prescribed drugs for inflammatory diseases. Another class of anti-inflammatory drugs is glucocorticoids such as prednisone and cortisone. In many countries in the tropics, people have limited access to these modern medicines and even when available, economic situation of majority of patients make it impossible for them to obtain those drugs.^[9] Nowadays herbal drugs are employed for treatment of inflammatory diseases rather than synthetic drugs. A number of studies have reported that extract of medicinal plants possess anti-inflammatory and analgesic activities. However, in most instances, the claims of therapeutic benefit of most of these medicinal plants are not supported by scientific evidence.

The plant *Erythrophleum ivorense* belongs to the family Fabaceae. The stem bark of the plant is used traditionally in the treatment of convulsive disorder, emesis, pain, swelling, small pox and as anthelmintic and laxative.^[10] Its anticonvulsant property has been reported^[11] Wakeel *et al.*, 2014. This study seeks to investigate anti-inflammatory and anti-nociceptive activities of *Erythrophleum ivorense* stem bark in experimental animals.

MATERIALS AND METHODS

Plants Materials

Fresh stem bark of *Erythrophleum ivorense* was collected from Iwo in Iwo Local Government area of Osun State, South West, Nigeria. The botanical identification and authentication of the plant was done in the herbarium unit, Department of Botany, Obafemi Awolowo University Ile-Ife. The voucher specimen of the plant was deposited at the herbarium unit of the institute (16878).

Preparation of Crude methanol extract (CME)

The stem bark of *Erythrophleum ivorense* (4.7kg) was dried and reduced to coarse powdery form using electric blending machine. Air-dried powder of *Erythrophleum ivorense* was extracted in 75% methanol. The filtrate was collected and concentrated using rotary evaporator. The sample was later freeze dried to get it in powdered form. The weight of the extract obtained was 430g. Crude methanol extract was prepared by dissolving in 2% dimethylsulphoxide (DMSO). The extract was administered intraperitoneally at doses of 5 to 20 mg/kg body weight to experimental animals.

Animal materials

Experiments were carried out using adult male mice (20-30g), maintained at 24±2°C under a 12-hour of light and 12-hour of dark cycle and with access to food and water

ad libitum. The animals were acclimatized for two weeks before experiment and were only used once throughout the experiments. All the experiments were conducted in accordance with the ethical guidelines on animal experimentation^[12], approved by Ladoke Akintola University of Technology, Ogbomosho. Animal care Unit Committee. Animals were fasted 12 hours prior experimentation

2.3 Analgesic studies

2.3.1 Acetic acid-induced writhing test Writhing in mice was induced according to a previously described method by Sulaiman *et al.*, 2008^[13], with slight modification. The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). Each mouse was given 0.6% aqueous solution of acetic acid and then placed in an observer box. The animals were pretreated for 30 minutes before acetic acid administration. Nociception was evaluated by counting the number of abdominal constriction for 20 minutes after administration of acetic acid. Percentage protection against abdominal constriction was taken as an index of analgesia. Acetylsalicylate (ASA, 150mg/kg, i.p), served as reference drug.

2.3.2 Hot plate test

Pain episode was induced by thermal stimulus as described by Hunskaar *et al.*, 1986.^[14] The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). The animals were pre-treated for 30 minutes; each mouse was placed in a hot plate maintained at 55±0.5°. Nociception was evaluated when the animal began to lick its hind paw or attempt to jump out of the hot plate. The time taken to lick the hind paw was taken as reaction time. Anti-nociceptive activity was expressed as the increased in reaction time. Morphine sulphate (5mg/kg, i.p) served as reference drug.

2.3.3 Formalin-induced paw lick test

The formalin assay is the most popular chemical assay of nociception. It entails the injection of a dilute solution of formalin into the surface of the rodent's hind paw, followed by scoring of stereotypical behaviours such as flinching, licking and biting of the affected hind paw.^[15] The behavior last for approximately one hour, with the early or acute phase (directly after injection) reflecting direct activation of nociceptors and late phase (15 or 20 minutes after injection) reflecting inflammation.^[15] In this experiment paw lick in rats was induced by formalin according to the method described by Hunskaar and Hole 1987.^[16] The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). Each mouse was injected at right hind paw with formalin (1%, 2ul). The animals were pre-treated for 30 minutes before injection of formalin. Nociception was evaluated when the animal began to lick its paw at 0-5 minutes

(early phase) and 20-30 minutes (late phase). Anti-nociceptive activity was expressed as the reduction in duration of paw lick. Morphine sulphate (5mg/kg, i.p) served as reference drug.

2.4 Anti-inflammatory Activity of extract

2.4.1 Carrageenin-induced paw lick

Pedal inflammation in rats was induced according to the method described by Winter *et al.*, 1962.^[17] The Rats were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). Oedema was induced by sub-plantar injection of 0.1ml of freshly prepared 1% carrageenan into the right hind paw of each rat. The animals were pre-treated for thirty minutes before sub-plantar injection (0 hour). Inflammation was evaluated by increased in paw volume, paw volume was measured at 0 and 3 hours after carrageenan injection using cotton thread. Anti-inflammation was expressed as

reduction or increased in percentage inhibition of paw volume. Indomethacin (5mg/kg, i.p) served as reference drug.

3.0 Statistical analyses

Data obtained from this study were expressed as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Newman-Keuls multiple comparison test. P-values less than 0.05 were considered statistically significant.

4.0 RESULTS

The result for the test of analgesic activity show that CME caused significant reduction ($p < 0.05$) in writhes induced by acetic acid, a dose-dependent effect was observed with percentage inhibition of 25.8, 83.7 and 88.7% for 5, 10 and 20mg/kg of the extract respectively. Aspirin, a reference drug produced 85.8% inhibition (figure 1).

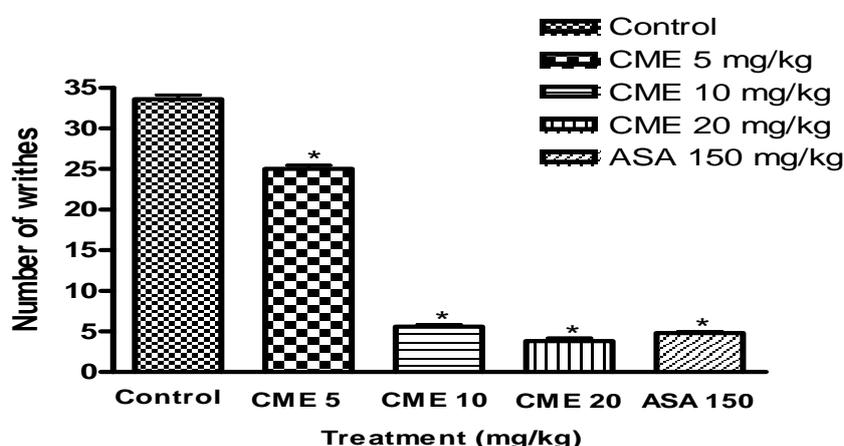


Figure: 1 Effect of Crude methanol extract (CME) of *E. ivorensis* on acetic acid induced writhing in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests.

In Table 1, it is shown that pre-treatment with morphine (5mg/kg) or with the extract (CME) of *Erythrophleum ivorensis* (5, 10 and 20mg/kg,) produced significant changes of paw licking time in the first phase of pain

response. In the second phase, a dose-dependent and significant ($p < 0.05$) reduction in licking time was observed in mice treated with CME (10 and 20mg/kg)

Table: 1 Effect of Crude methanol extract (CME) of *E. ivorensis* on formalin induced paw licking in mice. Latency of paw licks (s)

Pretreatment	Doses (mg/kg)	First phase	% inhibition	Second phase	% inhibition
Control	0	56.4±0.24	-	148.0±1.05	-
CME	5	38.2±0.45*	32.3	148.0±1.05	-
CME	10	35.6±0.40*	36.9	117.8±0.68*	20.4
CME	20	31.6±0.30*	44.0	67.4±0.58*	54.5
Morphine	5	33.4±0.25*	40.8	46.4±0.42*	68.7

First phase=0-5min after formalin injection; second phase= 15-30min. Data are mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests.

Due to analgesic effect observed in the first phase of formalin-induced paw lick, we decided to evaluate analgesic effect of CME using hot plate test, a model of central anti-nociceptive activity. CME significantly prolonged ($p < 0.05$) the reaction time of experimental animals to heat stimulus during hot plate test in a dose-

dependent manner. Morphine proved to be a potent analgesic, increasing the latency time within the

evaluation periods.

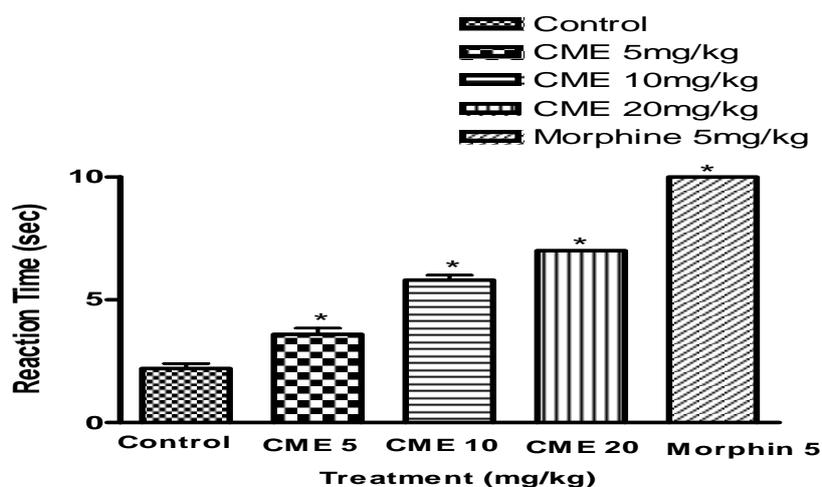


Figure: 2 Effect of Crude methanol extract (CME) of *E. ivorensis* on Hot plate test in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

Effect of carrageenan-induced Oedema in mice

The activity of CME on carrageenan-induced oedema in mice is shown on table 2. CME produced significant inhibition (p<0.05) of carrageenan-induced paw oedema. This effect was exhibited in a dose-related fashion.

Percentage inhibition produced by 5, 10 and 20mg/kg were 25.0, 41.7 and 58.3% respectively. Indomethacin, the reference drug produced a percentage inhibition of 68.7%.

Table 2: Effects of CME of *E. ivorensis* on carrageenan-induced rat paw oedema at 3 hours (peak oedema period)

Pretreatments	Doses (mg/kg)	Paw sizes (cm)		% inhibition
		before carrageenan	after carrageenan	
Control	0	2.0±0.20	3.2±0.50	0
CME	5	2.0±0.20	2.9±0.40*	25
CME	10	2.0±0.20	2.7±0.32*	41.7
CME	20	2.0±0.20	2.5±0.41*	58.3
Indomethacin	5	1.9±0.21	2.3±0.25*	68.7

DISCUSSION

Acetic acid mouse writhing is widely used animal model for routing screening of compound with peripheral analgesic activity.^[18,19] The writhing response is considered to be a visceral inflammatory pain^[18, 20] Acetic acid is a chemical irritant that produces tissue necrosis of the peritoneal region accompanied by the release of chemical mediators such as bradykinin, prostaglandin, histamine, substance P, vasoactive polypeptide, which cause pain either by activation or sensitization of nociceptors that encode tissue injury,^[18, 21] whilst the hot plate or tail immersion model of pains is generally used to detect centrally acting analgesics.^[16] CME produced dose-dependent and significantly (p<0.05) anti-nociceptive effects in chemically induced nociceptive pain stimuli in mice. The positive control group with ASA (150mg/kg) also manifested significant reduction in number of writhes but the inhibitory effect exhibited by CME against nociceptive action of acetic acid in mice may suggest the presence of

phytochemically active substances with analgesic property. In addition, it have been shown elsewhere that centrally and peripherally acting drugs such as morphine and aspirin are able to inhibit the inflammatory pain induced by acetic acid.^[22, 13] Therefore, the present results of acetic acid-induced abdominal constriction test strongly suggest that the mechanism of CME may be linked partly to inhibition of lipooxygenase and or cyclooxygenase in peripheral tissues, thereby reducing prostaglandin synthesis and interfering with the mechanism of transduction in primary afferent nociceptors. This suggestion further supported by the finding that CME inhibits the nociceptive (inflammatory pain) behavior produced by formalin in mice. The mechanism of analgesic effect of extract in acetic acid-induced writhing could be due to the blockade of the effect or the release of endogenous substances that excites pain nerve endings similar to that of indomethacin and other NSAIDs.^[23]

Formalin is used as chemical noxious stimuli to trigger pain. This test was normally used to study both central as well as peripheral analgesic activity.^[24, 25] Injection of formalin is associated with the neurogenic pain during early phase followed by the pain due to inflammation during the late phase.^[16, 26] The neurogenic pain is centrally mediated and is attributed to the direct stimulation of nociceptive primary afferents nerve fibres and the release of pain mediators such as kinin, histamine and serotonin. The inflammatory pain is peripherally mediated and it is due to peripheral release of chemical pain mediators that sensitize or activate nociceptors such as prostaglandin.^[25] The peripherally analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are only effective against inflammatory pain produced by formalin.^[25, 27] In contrast, the centrally acting analgesic drugs such as morphine inhibit both the neurogenic and inflammatory pains caused by formalin.^[16, 28, 26] The inhibitory effect demonstrated by CME against neurogenic and inflammatory pains may suggest peripheral and central analgesic actions similar to morphine. However, further study is needed to identify the active principle(s) and the mechanism underlying the analgesic effects of CME.

The peripheral analgesic effect of CME in the present study is strongly supported by the results obtained from hot-plate test which is a preferential method to screen centrally acting opiate analgesic drugs.^[24, 29] Hot plate is a transparent glass cylinder used to keep the animal on the heated surface of the plate.^[30] The temperature of hot plate is set using a thermoregulated water-circulated pump. This hot plate test is also considered to be sensitive to drugs acting at the supraspinal modulation level of the pain response^[31], suggesting atleast a modulatory effect of the investigated extract. The time of latency or reaction time is defined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal licks its paw or jumps off to avoid pain.^[32, 33] CME (5-20mg/kg) did exhibit analgesic activity. Hence, the result of hot plate test supported the result of formalin-induced paw licks and affirmed the presence of centrally acting analgesic activity. However, it is not known whether the analgesic action is opioid-like in nature and or involves dopaminergic or other mechanism. The use of selective antagonist like Naloxone or metoclopramide might help in understanding the mechanism involved.

Inflammation is typically characterized by increased permeability of endothelial tissues and influxes of blood leucocytes into the interstitium resulting in oedema. Many different biological mediators' influences each step of inflammation cascade and typically anti-inflammatory agents exhibit therapeutic properties by blocking the actions of synthesis of some of these mediators.^[2] Carrageenin-induced paw oedema was taken as a prototype of exudative phase of inflammation. This oedema depends on the participation of kinins and polymorphonuclear leucocytes with their

proinflammatory factors including prostaglandins.^[34] The development of oedema in the rat paw after the injection of carrageenin has been described as a biphasic event.^[35] The initial phase starts immediately after the injection and reduces within one hour, and is attributed to the release of histamine and serotonin.^[4] The second phase of swelling which begin at one hour and remain through three hour is due to the release of prostaglandin-like substances.^[3] It has been reported that the second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. Generally NSAIDs strongly inhibit the second phase of carrageenin-induced oedema while some inhibit both phases. Indomethacin seems to inhibit both phases.^[35, 36] Some of the phytochemicals found in certain herbs and plants are reported to demonstrate pain and inflammation reducing properties.^[37]

The effective anti-inflammatory activity was observed with CME treated animals for three hours measurement. The ability of CME to suppress acetic acid-induced nociceptive and carrageenin-induced inflammation suggests a peripheral analgesic effect similar to NSAIDs. The significant anti-inflammatory effect shown by CME against pain associated with second phase of formalin test and reduced pain episodes elicited by acetic acid may suggest involvement of phytochemically active constituents with prostaglandin synthesis inhibitory properties. Flavonoids have been reported to produce several anti-inflammatory effects.^[37]

In conclusion, crude methanol extract of *E ivorensis* possesses analgesic and anti-inflammatory effects. However the result of this experimental animal study lends pharmacological credence to the suggested folkloric, ethnomedicinal uses of the plant in the management and control of painful and inflammatory conditions. There is a need for more precise studies to determine and separate the active compounds and elucidate the mechanism of action responsible for the central nervous system effects of *E ivorensis*.

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