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Analgesic Activity and Oral Acute Toxicity Profile of the Ethyl Acetate Fraction of the Stem Bark of *Entada africana* Guill. et Perr.

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Abstract

Entada africana (Fabaceae) is a leguminous tree used in traditional medicine throughout West-Africa for the treatment of many diseases such as fever, respiratory tract complaints, diabetes, hypertension, diarrheas among others. This study investigated the oral acute toxicity and analgesic profile of stem bark extract of the plant. The stem bark was extracted successively using n-hexane, ethyl acetate and methanol to obtain; n-hexane fraction (NHF), ethylacetate fraction (EAF) and methanol fraction (MF) respectively. The oral acute toxicity as well as the anti-pyretic capability of the EAF was evaluated using standard procedures. The extract was administered orally to wistar albino mice at graded doses with reference to the investigated lethal dose of the EAF. Normal saline and piroxicam were used as negative and positive controls respectively. The EAF of the stem bark of E. africana demonstrated comparative activity to those of piroxicam in a dose dependent manner. The acute toxicity test showed that the stem bark of the plant is relatively safe to use up to a dose of 3.8 g/kg^{-1} body weight. This showed that the stem bark of this plant has the potential of being use as analgesic agent.

Keywords - Analgesic, piroxicam, *Entada africana,* acute toxicity,

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Introduction

Pains associated with disease condition is of concern because of its involvement in virtually all human and animal diseases The use of non – steroidal anti-inflammatory drugs (NSAIDs) and opiates which are commonly used to treat pains have been established to cause gastric lesions, tolerance and dependence (Dharmasiri *et al.*, 2003; Park *et al.*, 2004) and as a result attention is now being focused on the investigation of the efficacy of plant-based antipyretics since they are expected to be cheap, relatively available and have fewer side effects compared to the synthetic drugs used to treat pain. Natural products holds a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Hostettmann, 1987) as well as allow for the design and rational planning of new drugs, biometric synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann, 1991).

According to WHO, about 80% of the world population still rely mainly on herbal remedies for the treatment of one disease condition or another (Dharmasiri *et al.*, 2003; Li *et al.*, 2003; Kumara, 2001).

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.*, 2006). The medicinal value of these plants lies in their phytochemical component, especially the secondary metabolites such as alkaloids, tannins, anthocyanins, anthraquinone derivatives, flavonoids and other phenolic compounds (Anyasor *et al.*, 2011; Edeoga and Gomina, 2000).

Entada africana commonly known as "Tawatsa" , "Dorot" and "Ogurobe" in Hausa, Arabic and Yoruba languages belongs to the Fabaceae family. It is a well known leguminous tree used in traditional medicine throughout West-Africa in the treatment of many diseases such as fever, respiratory tract complaints, diabetes, hypertension, diarrheas (Occhiuto *et al.,* 1999). All part of the plant are being used especially its bark and leaves. Many studies have reported the wound-healing, haemostatic, anti-rheumatism, anti-

inflammatory, antibacterial, antileishmanial, hepatoprotective, fever, stomach ache, respiratory tract disorders, skin eruptions, rheumatism, cataract, dysentery, diabetes, hypertention as well as its protection against CCl4-induced liver damage (Orwa *et al.,* 2009, Berit, 2011, Occhiuto *et al.,* 1999, Beritsmestard, 2011, Nacoulma, 1996). In spite of its numerous applications in traditional medicine, there is very scanty scientific work on its antipyretic potential and oral acute toxicity profile. Therefore, this work was designed to investigate the antipyretic and acute toxicity profile of ethyl acetate extract of stem bark of this plant.

Materials And Methodology

Plant materials collection, preparation and identification

Fresh *E. africana* stem bark was collected in April, 2016 from Jabo town of Tambuwal Local Government area, Sokoto State North Western-Nigeria. They were washed with water to remove earthy impurities, identified and authenticated at the Herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University Sokoto where a herbarium specimen was deposited and a voucher number PCG/UDUS/FABA/0014 issued. They were then air dried for three weeks, powdered with the aid of a clean mechanical grinder and stored in an air tight glass container until use.

Chemicals

All the solvents, reagents and standards were purchased from Sigma Aldrich (France) and were all of analytical grade.

Extraction and Fractionation

5.2 kg of the powdered stem bark was macerated for 72 hours successively using solvent of increasing polarity starting with n-hexane, ethyl acetate and methanol. The solvents were decanted and filtered with Whatman filter paper. The filtrates were concentrated under reduced pressure at 45°C in a rotator evaporator (Stuart RE 300) and dried at room temperature to constant weight to obtain the n-hexane fraction (NHF), ethyl acetate fraction (EAF) and methanol fraction (MF) respectively. All the concentrated fractions were stored at 4°C till use.

Experimental Animals Procurement and Treatment

Swiss albino mice (25-36 g) of either sex were obtained from the Animal House Facility, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. All experimental procedures were reviewed and approved by the Faculty of Pharmaceutical Science Animal Ethics Committee with clearance number PTAC/Ea/OT/001-18 issued.

Acute Toxicity Studies

Prior to the experiment, the animals were fasted overnight but had free access to water *ad libitum*. The animals were fed with standard laboratory feeds and clean water during the experimental period. The extract was administered intra-peritoneally. The procedure was divided into 2 phases. The first phase had 12 mice sorted into groups IA, IIA IIIA and IVA with 3 mice in each group. Groups IA, IIA and IIIA received 10, 100 and 1000 mg/kg of extract respectively. The second phase had 4 groups IB, IIB, IIIB and IVB with 3 mice in each group receiving 1600, 2900 and 5000 mg/kg of the extract respectively. Groups IVA and IVB the control groups received 5 ml of tween-80 each the control vehicle for the extract preparation. The median lethal dose (LD_{50}) was calculated using the following formula:

Ld_{50}=

Table 1: Analgesic effect of *E. africana* on acetic acid induced writhing in mice

Groups	Doses	Mean Number of Writhing/10 min	Inhibition (%)
Normal Saline	1 ml/kg	13.00± 1.7	-
Acetic acid + Piroxicam	10 mg/kg	6.60± 0.2*	58.40
Acetic acid + EAF	125 mg/kg	7.80± 2.6*	40.00
Acetic acid + EAF	250 mg/kg	5.60± 1.6*	49.20
Acetic acid + EAF	500 mg/kg	5.40± 2.6*	56.90

Each value represents mean ± SEM. *$P<0.05$ is significant when compared with the negative control; n=5.

Acetic acid-induced abdominal writhing is a visceral pain model normally used to evaluate the peripheral analgesic effect of drugs and chemicals since the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway (Gupta *et al.*, 2005; Sawadogo *et al.*, 2006). The result showed that all the doses produced significant ($P < 0.05$) analgesic effect comparative to piroxicam the standard drug used for the study. This could be attributed to its anti-inflammatory effect since the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism (Sawadogo *et al.*, 2006; Franzotti *et al.*, 2002). The analgesic activity of the EAF demonstrated might be linked to the presence of secondary metabolites present in the plant since steroids, flavonoid, saponins and tannins have been reported to demonstrate analgesic activity (Das *et al.*, 1989; Pateh *et al.*, 2009).

Conclusion

The findings of the present study have demonstrated that the EAF of *E. africana* is a potent antipyretic agent and hence justify its use in traditional medicine to treat painful conditions. Its analgesic agent is in a dose-dependent manner. Based on the results obtained from the acute toxicity study, it can be concluded that the ethyl acetate extract of *E. africana* is not toxic in mice. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extracts and its active compound(s).

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