Anti-nociceptive and anti-inflammatory activities of extract of *Anchomanes difformis* in rats

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Abstract: *Anchomanes difformis* is a tropical plant that has been used in folklore to treat diverse complications. The leaf extract of *A. difformis* was investigated for possible anti-nociceptive and anti-inflammatory effects in albino wistar rats. In these independent studies, two sets of twenty five rats were divided into five groups of five rats per group. Formalin induced pain in rats was used to investigate the anti-nociceptive effect of the extract. The extract was administered orally in the treated groups at doses 200, 400, 800 and 1600 mg/kg with aspirin serving as the positive drug control while the normal control group was not given any extract but water. Studies were also carried out on the egg albumin induced anti-inflammatory activity in rats by inducing oedema on the left hind paw. The result showed a significant inhibition ($p<0.05$) on the later phase (800mg/kg) of formalin pain induction in rats; similarly, a significant ($p<0.05$) anti-inflammatory activity was observed at 60, 90 and 120 minutes. The study thus validates the ethnomedicinal usage of *A. difformis* in the treatment of pain and inflammation.

Keywords: *Anchomanes difformis*; araceae; anti-inflammatory; anti-nociceptive

INTRODUCTION

Inflammation is a common underlying factor that contributes to the aggravation of a wide variety of disease states which includes: asthma, arthritis, and cardiovascular disease which represent a serious health problem. Inflammatory diseases treated currently with steroidal and non-steroidal anti-inflammatory drugs exert their effects by inhibiting the metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways (Insel, 1996). Both steroidal and non-steroidal anti-inflammatory drugs currently used as anti-inflammatory drugs are known to exhibit some levels of toxicity effects. Because of this effect, compounds with less toxicity potential are needed. Thus, the search for natural products from plant origin having protective properties and possessing minimal side effects is largely becoming the final hope for mankind. *Anchomanes difformis* (Blume) Engl. belongs to the family of Araceae and it is native to some West African countries such as Nigeria, Ghana, Ivory Coast, Senegal, Sierra Leone and Togo. In South Western Nigeria, it is popularly called ìgo lángbòdó, ògìrìòsákó (Morten, 1961). The stem, leaf and tuber extracts of *A. difformis* were reported to contain alkaloids, tannins and saponins (Oyetayo, 2007). The phytochemicals in the rhizome extracts consist of phlebotannins, terpenoids and glycosides while the leaf extracts contained steroids but not reported in rhizome extracts among other (Abah *et al.,* 2011). The oil extract possesses antibacterial activity against *S. aureus, B. subtilis, K. pneumoniae and P. aeruginosa*. The antifungal activity has also been reported in the stem extract of *A. difformis* (Osho and Adetunji, 2010). Almost all the plant parts have been employed as purgatives; similarly, it is used for the treatment of dysentery, kidney pains, oedemas, urethral discharge and jaundice (Akah and Njike, 1990; Kerharo and Bouquet, 1950). The water and methanolic extracts of *A. difformis* exhibited antitrypanosomal activity (Bero *et al*., 2011) but does not possess antileishmanial nor antiplasmodial activities (Bero *et al*., 2009). The research was carried out to examine the potential anti-inflammatory and anti-nociceptive properties of ethanolic extract of *A. difformis* in order to validate its enthnobotanical claims in Nigeria.

MATERIALS AND METHODS

Plant collection

Anchomanes difformis leaves were obtained from Atan, Ado-Odo/Ota, Local Government, Ogun State, Nigeria in the month of November, 2011. The plant was authenticated by Dr. A.C. Omonhinmin of the Department of Biological Sciences, Covenant University, Canaanland, Ota, Nigeria.

Preparation of plant extract

The procedure of Adebayo *et al*. (2011) was employed for the extraction. The fresh leaves of *A. difformis* were

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washed, dried and grinded into powder using blender. The powdered material (226 g) was extracted using 95% ethanol solvent in Soxhlet apparatus. The extract was concentrated using rotator evaporator and gave a greenish mass, weight 42.12g representing 18.6% yield.

Experimental animals

Adult Wistar rats (150-210g) and Swiss albino mice (18-25g) of either sex were used in the experiments. These rats were received from the animal house of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. They were subsequently maintained under standard laboratory conditions and fed with formulated feeds from NIPRD and provided with drinking water *ad-libitum.* Animals were allowed to acclimatize for three weeks. The animal protocol and ethics of the institute were strictly adhered to. This is in consonance with the international principle of animal handling guideline procedures (NIH, 1985).

PHARMACOLOGICAL EVALUATION

Acute toxicity study

The acute toxicity was estimated in mice following the procedure described by Lorke (1983) with some modifications. The evaluation of lethal dose (LD_{50}) in mice was investigated in two phases. Mice in the first three groups were given the extract (*A. difformis*) orally at graded doses of 10, 100, 1000 mg/kg. This is to be able to know the range where the LD_{50} would fall. Similarly, four groups of three mice each were later administered with the extract at graded doses of 1600, 2900, 3600 and 5000 mg/kg in the second stage. Subsequently, the animals were observed for symptoms and signs of toxicity after 24 h. The number of deaths in each group within 24 h was noted and the net LD_{50} values were computed as the geometric average of the highest non-lethal dose (where no death occurred) and the lowest lethal dose (where there was occurrence of death).

Formalin induced nociception in rats

The study was carried out on albino Wistar rats by employing the techniques described by Akuodor *et al.*, 2010; Tjolsen *et al* (1992); Dubbisson & Dennis (1997). Albino rats (30) were separated into six groups of five rats each per group. Animals in group I which served as negative control group were given water only and without the extract; animals in group II received standard drug, aspirin (ASA: 150 mg/kg), and animals in groups III, IV V, and VI were orally administered with 200, 400, 800 and 1600 mg/kg of the extract. One hour after the treatment, 0.25% formalin was subcutaneously injected into the sub planar surface of the left hind paw. The animals were subsequently kept in a chamber and observed for 60 min. The severity of nociception; response was based on the following scale: 0, rats walked and stood firmly on injected paw; 1, the injected paw was favoured or partially elevated; 2, the injected paw clearly lifted off floor; 3, the rat licked, chewed and shook the injected paw. Anti-nociceptive effects were determined in two phases: early phase (first 0-5 minutes) with a relatively free pain period for (10 minutes) and at every 5 minutes between the next (15 to 60 minutes) interval for late phase.

Anti-inflammatory study

The study was determined by adopting techniques described by Akah and Nwabie (1994) and Winter *et al* (1963). Albino rats (30) were grouped into five rats each per group and were treated as follows: animals in group I served as negative control and given water but no extract; rats in group II were treated with acetyl salicylic acid aspirin, ASA) (150 mg/kg body weight) and therefore served as the positive control. Similarly, animals of groups III, IV, V and VI were orally administered with 200, 400, 800 and 1600 mg/kg body weight of *A. difformis* respectively. Oedema was induced by injecting rats with of 0.1 ml of new raw egg albumin in the left hind paw after 30 minutes of post drug administration. Oedema size was evaluated by using a digital letica plethysmometer (LE7500, Spain), with results taken at 30 minutes intervals i.e.: 0, 30, 60, 90, 120 minutes after albumin administration.

STATISTICAL ANALYSIS

Data are presented as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package, Graph pad prism 5 was used for data analysis.

RESULTS

Acute toxicity tests

The toxicity signs exhibited by the mice are paw licking, rigorous shaking of tail, clustering. The oral lethal dosage (LD_{50}) of *A. difformis* in mice was estimated to be greater than 5000 mg/kg.

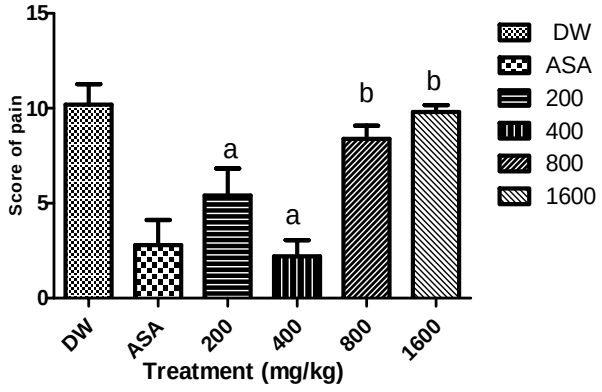

Fig. 1: Effect of *A. difformis* on formalin induced nociception in albino rats.

Values represent mean ± S.E.M of 5 replicates. [a]$p<0.05$, significantly different from DW (distilled water); [b]$p<0.05$, significantly different from ASA (aspirin).

Formalin-induced nociception assay

The result showed that the ethanolic extract of *A. differmis* reduced formalin paw nociception and paw licking in groups treated with 200 and 400 mg/kg bw but was significantly ($p<0.05$) increased at 800 and 1600 mg/kg bw when compared with the positive control drug (aspirin) (fig. 1).

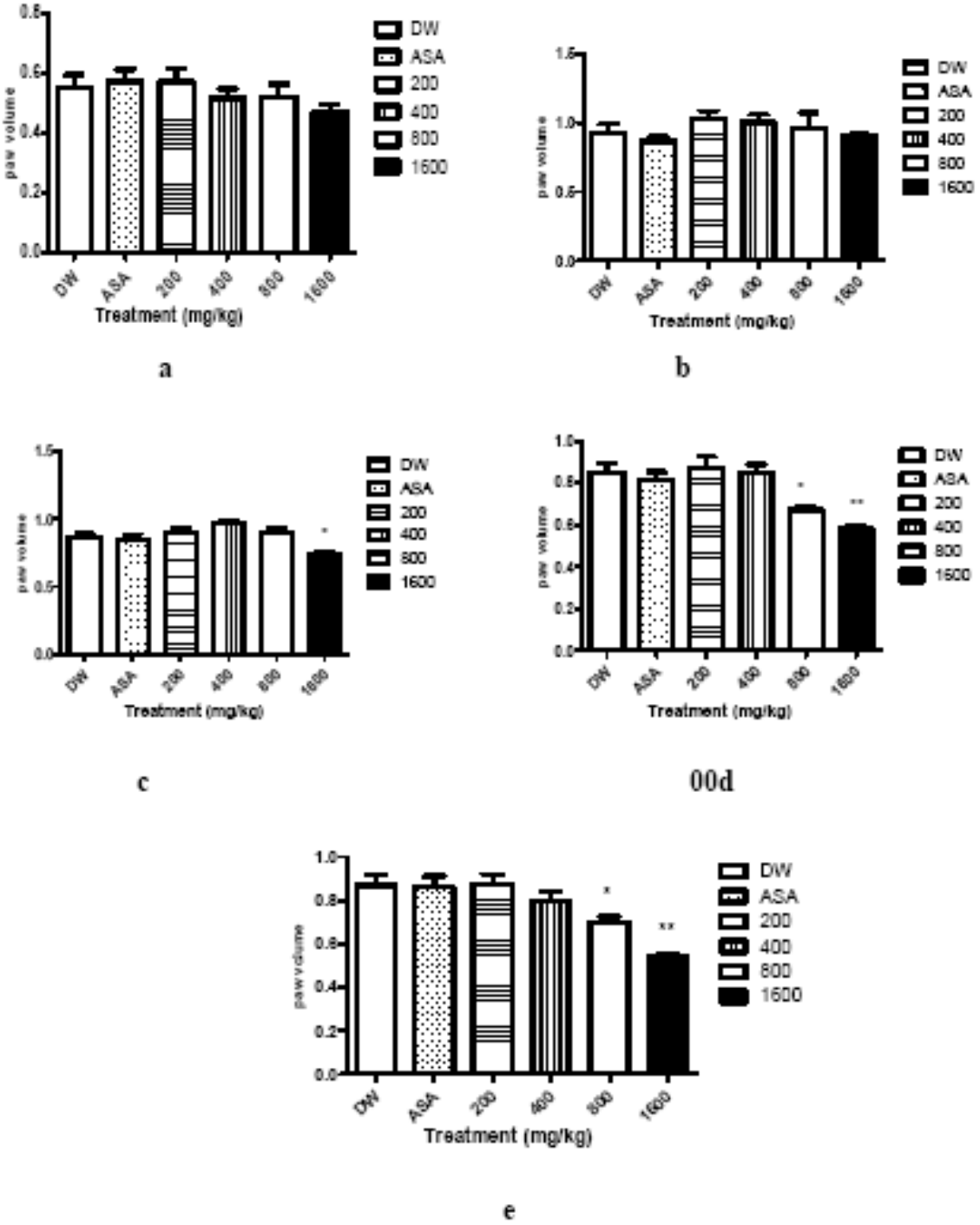

Fig. 2(a-e): Effect of *A. differmis* on albumin induced inflammation in albino rats

Values represent mean ± SEM of 5 replicates. *$p < 0.05$

Figs. 2a-e show the effects of albumin induced inflammation in albino rat at 0, 30, 60, 90 and 120 minutes respectively

Inflammatory assay by albumin induction

The results obtained indicated that there was no significant anti-inflammatory effect in the groups treated with 200 and 400 mg/kg bw within the time frame as compared with the control groups (fig. 2a-c). However, the groups treated with 800 and 1600 mg/kg bw of extract of *A. difformis* exhibited significant inhibition ($p<0.05$) on the paw volume after 60, 90 and 120 minutes (fig. 2c-e) [AA1].

DISCUSSION

Acute toxicity tests are often undertaken to know the concentrations of chemicals or materials that can produce a harmful effect on a group of test organisms within a short-term duration under controlled conditions (Rand and Petrocelli, 1985). The extract of *A. difformis* showed an LD_{50} greater than 5000 mg/kg bw in mice indicating that it is well tolerated in the animals and may not pose any risk within these concentrations. Many studies have revealed that inflammation and pain are associated with disease of various clinical conditions like arthritis, vascular and cancer diseases (Coderre *et al.*, 1990). Moreover, many medicinal plants have been found to alleviate inflammation and pain in *in vitro* and *in vivo* systems. *A. difformis* is a medicinal plant commonly used traditionally for pain relief and other medicinal uses. The study investigated the effect of *A. difformis* on inflammation and taking into account the correlation between analgesic and anti-inflammatory effects. Extracts and pure compounds from medicinal plants have been screened for their ability to modulate the expression of pro-inflammatory signals and thus could possibly serve as anti-inflammatory lead agents. The following phytochemical compounds have been employed as anti-inflammatory agents; polyphenols, terpenes, catechines, flavonoids, quinines, alkaloids, etc (Shailasree *et al*., 2012). The mechanism of anti-inflammatory activity for these phytochemicals has been reported in literatures. One of such is through the inhibition of NF-kB activation and down-regulation of the expression of inflammatory enzyme markers such as., 5-LOX, COX-2, and MMP-9 (Khanna *et al*., 2007). The result from phytochemical screening of *A. difformis* as conducted by Abah *et al* (2011) indicated that the plant contains biological active substances including tannins and flavonoids with potential values for the treatment of inflammatory and painful conditions. The animals that received the higher doses of 800 and 1600 mg/kg body weight of the extract of *A. difformis* were found to significantly inhibit albumin induced inflammation after 60, 90 and 120 minutes. The inhibition may have been initiated by the presence of one or some bioactive compounds earlier mentioned. In rheumatoid arthritis, flavonoids were reported to inhibit the release of chemical mediators through the histamine while serotonin reduces the symptoms. These were thought to be mediated through decreased monocyte infiltration and fibroblast proliferation, blocked TNF-α and inhibition of COX (Majumdar *et al*., 2008). The mode of action of the anti-inflammatory potential of the plant extract may have probably followed the same pattern. Our finding is in consonant with the study conducted by Das *et al* (2009) on the anti-inflammatory effect of *Xeromphis spinosa* extract in carrageenin-induced paw edema rats which exhibited significant effect after 4 hours of administration. Similarly, the methanolic extract of *Artemisia absinthium* exhibited significant anti-inflammatory effect in mice. The extract showed a delayed anti-inflammatory response and was suggested to be due to the delayed absorption of the plant extract (Ahmad *et al*., 1992).

Pains can be stimulated by injection of irritants into the subcutaneous cavity of rats. The animals react with vigorous licking of paws. The formalin model is usually considered for chronic pain induction (Dubuisson and Dennis, 1977). *A. difformis* significantly inhibited vigorous paw licking in the succeeding phase in response to formalin induction. The rats dosed with 200 and 400 mg/kg body weight of the extract significantly reduced nociception which was quite comparable to the positive control, aspirin. The result obtained is in tandem with the work of Hosseinzadeh & Younesi (2002) where the effect of ethanolic and water maceration extracts of *Crocus sativus L.* were examined for antinociceptive activity in mice. Antinociception study was tested using the hot plate and writhing tests. The extracts exhibited antinociceptive activity against acetic acid induced writhing. In a similar study, the hydroalchoholic extract of *Thymus vulgaris* showed a significant effect against pain in three antinociceptive models examined in mice: formalin, tail flick and hot plate tests. The animal dosed with 500 mg/kg of this extract was found to be more potent than the control drugs used. Opioid, serotoninergic and cholinergic receptors were implicated as possible mechanism of mediating the antinociceptive effects of this plant (Taherian *et al*., 2009). The antinociceptive property of *A. difformis* may also possess the same pattern.

But the experimental animals offered two discrete nociceptive behavioral phases, which perhaps involve dissimilar stimuli. The first phase which was initiated immediately after formalin injection lasted between 0 - 5 minutes resulting from chemical stimulation of nociceptors. This may be due to direct effects on nociceptors, experimentally available data showed that formalin predominantly stirs activity in the C-fibres and not the A- afferents, this phase can be inhibited by centrally acting analgesics. The second phase was initiated 15 - 30 minutes after formalin administration and may likely depend on a peripheral mechanism as well as a centrally mediated one. The late (second) phase seems to be due to an inflammatory response partially mediated by prostaglandins and can be suppressed by peripheral drugs such as NSAIDs and steroids, as well as the centrally acting drugs (Viana *et al*., 2003). The effects of *A.*

differmis was significant only in the second phase, demonstrating a process related to the inflammatory process (Ferreira *et al*., 1971).

CONCLUSION

The study clearly showed that *A. differmis* exhibited some degrees of inhibition against albumin induced inflammation at a later phase in rats while also showing significant activity in formalin induced nociception in rats, thus validating its use in ethnomedicine. Although, flavonoid and some other phytochemicals have been implicated in mediating these activities, the exact mechanism of this plant in initiating anti-inflammation and anti-nociception in rats could be further investigated.

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