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Anti-Inflammatory Properties of the Fruits of *Allanblanckia floribunda oliv. (Guttiferae)*

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Abstract: The present study aims to investigate the anti-inflammatory properties of the fruits of *Allanblackia floribunda* Oliv. [Guttiferae]. The fruits are traditionally used in the treatment of anti-inflammatory conditions. The methanolic extracts of the fruits (100-400 mg/kg, p.o) inhibited carrageenan induced paw oedema in rats in a dose dependent manner. At 400 mg/kg *A. floribunda* (AF) produced an inhibition of 76.9% compared to 64.3% for indomethacin (10 mg/kg). Acetylsalicylic acid (100 mg/kg - ASA) did not show significant activity in the inhibition of the neurogenic phase, but was more active than *A. floribunda* in the inhibition of the inflammatory phase of formalin-induce pain. *A. floribunda* was not active as an anti-inflammatory agent up to 2 h in the histamine-induced rat paw oedema but a significant reduction in paw size was observed after 2 h. This suggests that AF was more active in the later phase of the inflammatory process. AF also reduced hot plate latency in a dose dependent manner. Hot plate latency of 26.8 s was recorded for AF at 400 mg/kg compared to 18.12 s for normal saline and 30.6 s for ASA. LD_{50} value was determined in Swiss albino mice as 20 g/kg and so can be regarded as relatively safe.

Key words: Allanblackia floribunda · Anti-inflammatory · Rat paw oedema · Analgesic

INTRODUCTION

Conventional drug treatments are limited in their effectiveness in managing the incidence and outcome of many inflammatory diseases. They also present a significant number of side-effects in patients and recently, it has been shown that non-steroidal anti-inflammatory agents may even slow down the healing process. There is therefore an urgent need to find safer and more effective drug treatments.

Allanblackia floribunda is an evergreen tree found in the rainforest. Its fruits are large, up to 30 cm long and 10 cm in diameter containing seeds in a translucent mucilage. A decoction of the whole fruit is used in Ivory Coast to relieve scrotal elephantiasis. In Nigeria and Ghana it is used to relieve toothache and rheumatism. The decoction of the bark is taken for dysentery and mouthwash in Gabon. In Congo, it is taken for stomach ache and a decoction of the bark or the leaves is also taken for cough, asthma, bronchitis and other bronchial infections. The traditional uses indicate possible anti-inflammatory and antimicrobial activity. The decoction of the leaves and fruits have also been reported for use in the treatment of malaria and toothache [1,2]. All parts of the plant are used traditionally in the treatment of smallpox, chickenpox and measles indicating possible antiviral activity. The fatty substance of the seeds is mildly purgative.

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MATERIALS AND METHODS

The Plant Materials: The fruits of *Allanblackia floribunda* (Guttifereae) were obtained from Oke-igbo in South Western Nigeria. Botanical identification were performed at the Forestry Research Institute of Nigeria (FRIN) by Mr. O.M. Awoleye and given a voucher number (FHI107929).

Preparation of Extracts: The fruits were cut into pieces and dried at 40°C, grinded and soaked in methanol (Analar grade) for three days. The crude extract was filtered off and concentrated using a rotary evaporator. 60 g of the powdered fruit of Allanblackia *floribunda* yielded 11.65 g of methanol extract (19.4% yield).

Experimental Animals: The animals used for the anti-inflammatory studies were Sprague-Dawley rats of both sexes weighing 120-150 g and Swiss albino mice weighing 25-30 g supplied by the Animal House of the College of Medicine, University of Lagos. They were fed on normal rat feed obtained from Pfizer Livestock Feeds limited, Ikeja. Animals were kept in cages with solid floors covered with wood shavings Animals in a photo period-controlled environment (12 h light-dark cycle). They were given food and water *ad libitum.*

The Drugs: Acetylsalicylic acid (ASA) and Indomethacin were used as reference drugs for the analgesic and anti-inflammatory tests at a dose of 100 mg/kg and 10 mg/kg respectively.

Anti-Inflammatory Studies

Carrageenan-Induced Rat Paw Oedema: Acute inflammation in the rats were produced according to the method described by Winter *et al* [3]. Five groups on rats each containing five animals per group were used for the study. Group 1 served as the control group receiving normal saline 5 ml/kg, animals in group 2 were given 10 mg/kg indomethacin orally while groups 3,4 and 5 received the plant extracts at doses of 100, 200 and 400 mg/kg respectively. Animals were given a saline solution, indomethacin and the appropriate dose of the extract depending on the group, 1 hr before administration of an intradermal injection of carrageenan (0.1 ml of a 1% solution in 0.9% saline solution) into the plantar region of the right hand paw. The paw size was measured before injection of carrageenan and every hour for a period of 6 h. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. The average increase in paw size of each group was calculated and compared with the control (normal saline) and indomethacin groups. The percentage inhibition was calculated thus:

$$\% \text{ inhibition} = \frac{(S_t\text{-}S_0) \text{ control} - (S_t\text{-}S_0) \text{ treated}}{(S_t\text{-}S_0)\text{control}} \times 100$$

Where S_t = the mean paw size for each group after carrageenan treatment

And S_o = the mean paw size obtained for each group before carrageenan injection.

Histamine-Induced Rat Paw Oedema: Five groups of rats, each containing five animals per group were used for the study. Group 1 served as the control group receiving normal saline 5 ml/kg, animals in group 2 were given indomethacin 10 mg/kg orally while groups 3,4 and 5 received the plant extracts at doses of 100, 200 and 400 mg/kg respectively. Animals were given saline solution, indomethacin and the appropriate dose of the extract orally, depending on the group, 1 hr before administration of an injection of histamine (0.1 ml of a 1% solution in 0.9% saline solution) into the plantar region of the right hand paw. The paw size was measured before injection of histamine, at 30 mins after injection and every hour for a period of 6 h. The paw oedema was measured as described above [4-6].

Analgesic Studies

Formalin-Induced Pain: Five groups of five animals were used. Each group of mice was treated with the plant extract (100, 200 or 400 mg/kg), normal saline solution or ASA (10 mg/kg) orally. One hour after treatment, all animals were injected with 20 µl of a 5% formalin in saline solution into the plantar surface of the left hind paw. Licking time was measured over 30 mins divided into two phases. The first phase was from time zero to 5 min after formalin injection and the second phase was from 20-30 mins after formalin injection. Percentage inhibition was obtained by using this formula:

$$\% \text{ decrease in paw licking time} = \frac{T_o - T_t}{T_o} \times 100$$

Where
T_o = mean licking time for the control group
T_t = mean licking time for the test group

Hot Plate Test: Twenty five Swiss albino mice were divided into 5 groups of five mice per group. Group 1 received normal saline, group 2 received ASA 100 mg/kg while groups 3,4 and 5 received 100, 200 and 400 mg/kg plant extract respectively p.o.. The mice were placed on top of a hot plate of 55 ± 0.7°C, one hour after the drug was administered. The time between placement and jumping was recorded as response latency. The reaction time was recorded for the control mice and for the animals pre-treated with plant extracts [7]. All the drugs were administered orally. The percentage increase in reaction time was calculated thus:

$$\% \text{ increase in reaction time} = \frac{T_t - T_o}{T_o} \times 100$$

Acute Toxicity Test: Thirty Swiss albino mice were divided into five goups. The extract was reconstituted in normal saline and administered orally as a single dose to groups at different concentrations (2, 4, 8, 16 and 32 g/kg). The animals were observed for a period of 48 h. The number of deaths recorded was expressed as a percentile and the LD_{50} was determined by probit test using the death percentage versus the log dose [8].

All the experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines.

Statistical Analysis: The data are expressed as mean ±S.E.M. and the Student's "t" test was used for comparison of the data of the control and standard groups. Probabilities of < 0.05 were considered as significant.

RESULTS

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 h. At a dose of 100 mg/kg AF produced a maximum inhibition of carrageenan induced inflammation of 53.8%. The inflammatory effect of carrageenan was no longer significant after 5 h at this dose. Maximum percentage inhibition of 69.2% and 76.9% were recorded at 200 and 400 mg/kg doses respectively. The inflammatory effect of carrageenan was no longer significant after 4 hours at these doses. Indomethacin 10 mg/kg p.o produced an inhibition of 64.2% and the inflammatory effect was no longer significant after 4 hours of treatment (Fig. 1).

The extract did not exhibit a significant anti-inflammatory effect up to 2 hr at all the doses tested in the histamine-induced rat paw oedema model. However a maximum inhibition of 20% after 4 h at a dose of 100 mg AF, 83% at a dose of 200 mg AF after 5 h and 100% at a dose of 400 mg AF after 3 h were exhibited in the

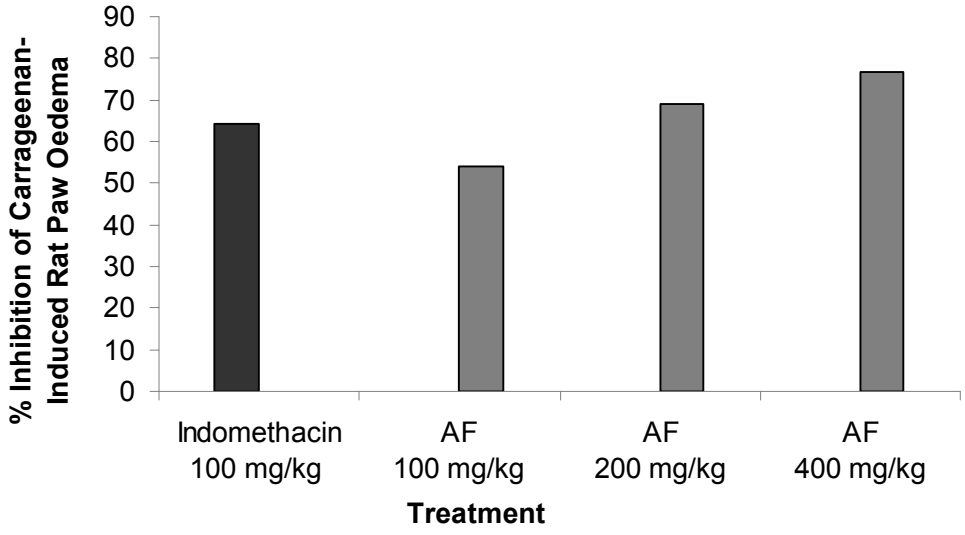

Fig. 1: Effect of the methanol extract of *Allanblackia floribunda* fruits on carrageenan-induced rat paw oedema in rats

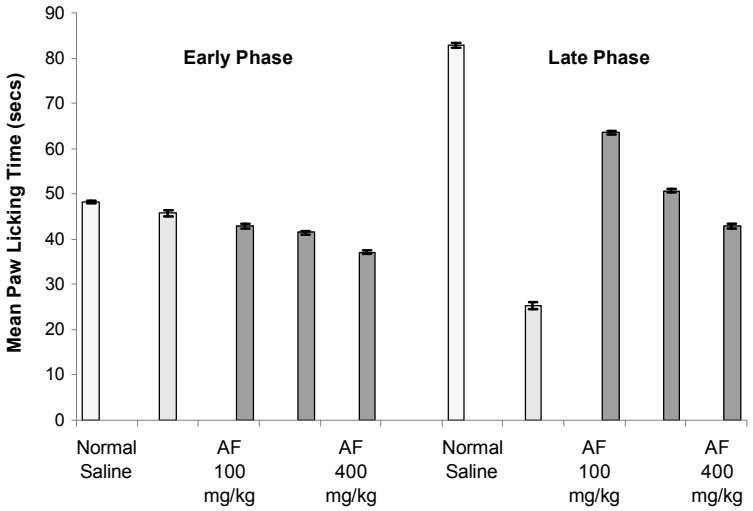

Fig. 2: Effect of methanol extract of *Allanblackia floribunda* fruits on formalin-induced paw licking in mice

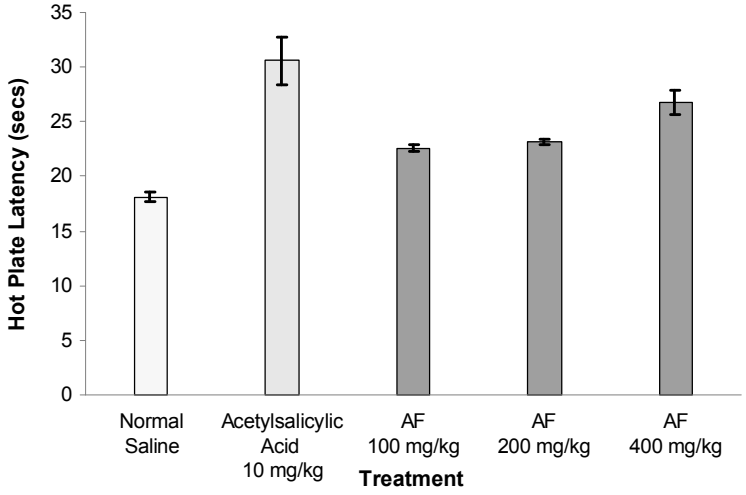

Fig. 3: Effect of methanol extract of Allanblackia floribunda fruits on hot-plate latency in mice

histamine-induced rat paw oedema while indomethacin 10 mg/kg exhibited a maximum of 100% inhibition after 3 h in this model.

The response time of the control mice in the first and second phases in the formalin induced pain model were 48.2 ± 0.27 s and 82.8 ±0.51 s respectively. Only a slight reduction in licking time was noted for *A. floribunda* in the first phase. The response time were 42.8 ± 0.56 s and 41.5 ± 0.41 s for 100 and 200 mg AF respectively, representing 11.4 and 14% reduction in licking time compared to the control experiment. However at 400 mg/kg dose of AF the mean licking time was 37.1 ± 0.41 s (23.1% reduction compared to the control). Only a 5.5% reduction in licking time was recorded for ASA (45.7 ± 0.57 s). In the second phase, the mean licking time was reduced by AF in a dose dependent manner 63.6 ± 0.38, 50.7 ± 0.41 and 42.8 ± 0.57 respectively representing 23.3, 38.8 and 48.4% reduction respectively for doses of 100, 200 and 400mg/kg AF. Mean licking time recorded for ASA (100 mg/kg) was 25.0 ± 0.83 (69.8%) (Fig. 2).

The reaction time increased in the mice treated with the extracts of AF at all the doses tested in the hot plate test. The reaction time observed for ASA 10mg/kg was 30.6 ± 2.16 s while reaction times of 22.56 ± 0.3, 23.16 ± 0.3 and 26.76 ± 1.08 s were observed for 100 mg, 200 mg and 400 mg of AF respectively (Fig. 3). Percentage increase in reaction times were 68.8, 24.5, 27.8 and 47.7% for ASA, 100 mg, 200 mg and 400 mg AF respectively.

LD_{50} was determined as 20 g/kg from the acute toxicity test.

DISCUSSION AND CONCLUSIONS

The study establishes the anti-inflammatory activity of the methanol extract of the fruits of *Allanblackia floribunda* in the models used. Carrageenan-induced oedema is commonly used in animal models for acute inflammation and is believed to be a biphasic event [9]. The early phase (1-2 h) is attributed to the release of histamine and serotonin followed by a later phase of oedema due to production of bradykinin and prostaglandins. The second phase has been reported to be sensitive to both steroidal and non-steroidal anti-inflammatory agents [9]. In this study, the extracts did not show a significant anti-inflammatory effect in the early phase but showed significant effect at the later phases after 4-5 h. In the histamine model, the extracts also did not show a significant anti-inflammatory effect until after 2 h. The time of peak inflammation for histamine is 30 mins at which time the extracts did not exhibit any significant effect at all the doses tested. The results suggest that the extract acts at the later phase involving arachidonic acid metabolites possibly by the inhibition of cyclooxygenases.

The formalin test consist of two different phases, reflecting different types of pain. The early phase is due to direct effect on nociceptors and prostaglandins do not play an important role during this phase [10]. The late phase appears to be due to an inflammatory response with pain that can be inhibited by anti-inflammatory drugs. However studies have indicated that endogenous opoid-peptidergic and serotoninergic systems modulate both the early and late phases differently [11]. Centrally acting analgesics are also know to inhibit the two phases, in contrast to non-steroidal anti-inflammatory drugs like indomethacin that only inhibit the late phase [10]. In this study, the extract though slightly active in the first phase, showed better activity at the later phase of the formalin induce pain. However its effect on the early phase of the formalin induced pain was greater than that of acetylsalicylic acid. This further supports the anti-inflammatory action of the extracts with antinociceptive activity. The extracts also showed potent antinociceptive actions in mice by increasing the latency period in the hot-plate test. The acute toxicity test in mice suggests that the extracts are relatively safe.

ASA appeared to be more potent in the second phase of the formalin induced pain and in increasing the latency time in the hot plate test as may be expected, since the crude extract was used in the study. Isolation of the bioactive compounds with antinociceptive properties may reveal a very potent analgesic agent. Interestingly in the carrageenan-induced paw oedema model, indomethacin appeared to be less potent compared to 400 mg/kg dose of the crude drug extract. Isolation of the active constituent(s) of AF is likely to reveal very potent anti-inflammatory agent(s). This findings support the folkloric use of this plant as an anti-inflammatory/analgesic agent. Attempts are now been made in the isolation of bioactive agents through bioassay activity guided fractionation.

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