

Original Article

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *ARENGA WIGHTII* GRIFF.-AN ENDEMIC PALM OF WESTERN GHATS

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ABSTRACT

Objective: The present study aims to scientifically validate the anti-inflammatory and analgesic activities of *Arenga wightii*.

Methods: The stem pith was excised from mature palm, sliced into small pieces, shade dried and powdered. The powder was extracted with ethanol, concentrated under reduced pressure and the crude extract was referred to as AW. The anti-inflammatory and analgesic activity of AW was analyzed in Wistar rats and Swiss albino mice.

Results: The results revealed that the ethanolic extract of the stem pith of *A. wightii* showed a dose dependent anti-inflammatory and analgesic activity, which was comparable to the standards, indomethacin and acetyl salicylic acid respectively.

Conclusion: The results of the current study reveal that *A. wightii* possesses significant anti-inflammatory and analgesic activity.

Keywords: Anti-inflammatory, Carrageenan-induced paw oedema, Cotton pellet-induced granuloma, Acetic acid-induced vascular permeability, Acetic acid-induced writhing, Formalin induced nociception, Acetyl salicylic acid, Indomethacin.

INTRODUCTION

Inflammation has been regarded as a protective attempt to eliminate the harmful stimuli and activate the healing process by the organism. However, persistent inflammation leads to tissue damage and possibly failure of organs [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used medications in the world, due to their efficacy in reducing pain and inflammation [2].

Although the traditional NSAIDs are effective in relieving pain and inflammation, they are associated with adverse effects including alterations in blood pressure, hepatic injury, platelet inhibition and the significant risk of serious gastrointestinal and cardiovascular events in chronic use [3, 4]. Therefore increased attention has been focused on ethnological plants as they are affordable and have less toxicities and adverse effects [5].

Asia is endowed with the world's greatest palm diversity and also possesses the highest diversity of palm utilization. Palms represent the third most important plant family with respect to human use [6]. They have a long history of management for both subsistence and commercial products, many of which are deeply embedded in local cultures [7]. *Arenga wightii* Griff. (Arecaceae) locally known as 'Njettippana' or 'Kattuthengu', is an endemic monocarpic palm found in the dense evergreen forests of the Western Ghats of Kerala.

The pith obtained from the plant is used by various tribal communities residing in Kerala for the treatment of various ailments like jaundice, body aches, general weakness, painful urination, leucorrhoea, venereal diseases [8-12] and also as a source of food [13-15]. Since *A. wightii* pith is used for various ailments and it has not been previously scientifically validated, the present study was carried out to determine the anti-nociceptive and antioxidant activities of the ethanolic extract of *A. wightii* stem pith.

MATERIALS AND METHODS

Plant material

Arenga wightii was collected from Kannur, Kerala. It was identified and authenticated by Dr. N Mohanan, plant taxonomist of the Institute. A voucher specimen of the plant was deposited at the herbarium of the Institute (TBGT 57046, dated 30/04/2010).

Preparation of the plant extract

The pith was collected from the trunk of mature flowering plant of *A. wightii*, sliced into small pieces, shade dried and powdered. The powder was extracted with ethanol, concentrated under reduced pressure and crude extract was referred to as AW. This was then reconstituted in water to appropriate concentrations for further studies.

Animals

Wistar rats (175-200g) and Swiss albino mice (20-30 g), of either sex, obtained from the Institute's Animal House were used for the studies. They were housed under standard laboratory conditions and were fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (Registration No. 25-1/99/AWD 176/CPCSEA dated 29/09/1999).

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

Oedema was induced in rats according to the method of Winter *et al.* (1962) [16]. The animals were divided into five groups of six animals each and fasted overnight. Group 1, the control group was administered 1 ml distilled water, Group 2 received 1 ml indomethacin (10 mg/kg, standard), while Groups 3, 4 and 5 received various concentrations of the AW (125, 250 and 500 mg/kg respectively, p. o). 30 min after extract administration, 0.1 ml, 1% carrageenan (Sigma Chemical Company, USA) was injected into the right hind paw, under the plantar aponeurosis. The hind paw volume was measured plethysmographically just before and 3h after carrageenan injection. The difference in the paw volumes indicated the degree of inflammation.

Cotton pellet induced granuloma

The method described by Ismail *et al.* (1997) [17] was used with modification. The animals were divided into five groups of six animals each. They were fasted overnight and sterile cotton pellets (20±1.5 mg) were implanted subcutaneously on the back of anesthetized rats. Group 1, the control group was administered 1 ml distilled water, Group 2 received 1 ml indomethacin (10 mg/kg, standard), while Groups 3,4 and 5 received various concentrations

of the AW (125, 250 and 500 mg/kg respectively, p. o), once a day for seven consecutive days. On the 8th day, the rats were sacrificed and the cotton pellet excised, weighed and dried overnight at 60 °C. The dry weight was estimated.

Acetic acid induced vascular permeability

The method of Whittle (1964) [18] was used. The overnight fasted Swiss albino mice were divided into 5 groups of six animals each. Group 1, the normal control group received distilled water, p. o. Group 2 received 0.5 ml of indomethacin (10 mg/kg) p. o. Groups 3, 4 and 5 received various concentrations of AW (125, 250 and 500 mg/kg respectively) p. o. 1h after administration of the drug, 0.2 ml of 0.25% of Evans blue was administered intravenously through the tail vein. 30 min later, 0.6% acetic acid (0.5 ml) was administered intraperitoneally and after half an hour, each mouse was anaesthetized and given 5 ml of normal saline, intraperitoneally. The peritoneal cavity was massaged well and the peritoneal fluid collected after sacrificing the animals. The peritoneal fluid was centrifuged at 1000rpm for 20 min and the O D was read at 610 nm using a spectrophotometer (Agilent Cary 100 UV-Vis).

Analgesic activity

Acetic acid induced writhing

Analgesic response was assessed by the method of Koster *et al.* (1959) [19]. Swiss albino mice were divided into 5 groups of six animals each. Control group (Group1) received a single dose of distilled water (0.5 ml) orally and the standard group (Group 2) a single dose of 0.5 ml acetyl salicylic acid (25 mg/kg) orally. Groups 3, 4 and 5 received a single dose of AW (125, 250 and 500 mg/kg respectively, p. o). After 20 min, 0.5% acetic acid (0.25 ml) was administered intra-peritoneally to all the groups. The number of writhes per animal was counted for 30 min, starting 5 min after treatment with acetic acid.

Formalin induced paw licking

Swiss albino mice were divided into 5 groups of six animals each. Control group (Group1) received a single dose of distilled water (0.5 ml) orally and the standard group (Group 2), a single dose of 0.5 ml acetyl salicylic acid (25 mg/kg) orally. Groups 3, 4 and 5 received a single dose of AW (125, 250 and 500 mg/kg respectively, p. o). After 1h, 2.5% formalin (20 µl) was injected onto the ventral surface of the right hind paw of all the animals. The animals were observed from 0-5 min (neurogenic phase) and from 15-30 min (inflammatory phase). The time they spent licking the injected paw was recorded and considered as indicative of nociception [20, 21].

Behavioral and toxic effects

Toxicity studies were carried out by an acute toxic class method as described in OECD Guidelines No.423 [22]. Swiss albino mice of either sex weighing 20-25g were randomly distributed into 8 groups of 6 mice each. The overnight fasted mice were given a single oral dose of the extract at varying doses (AW 50, 100, 250, 500, 1000, 2500, 5000, 7500 mg/kg).

The animals were observed individually after dosing, continuously for the first 30 min and then periodically for 24h and daily thereafter for 14 days. All animals were observed for any signs of toxicity (such as change in body weight, colour of body surface, nature of movement etc.) during the study period.

Preliminary phytochemical analysis

AW was subjected to preliminary phytochemical analysis as per standard methods [23, 24].

Statistical analysis

All values are expressed as mean±Standard Deviation. The level of significance was analyzed by "One-way ANOVA with Dunnett's posttest performed using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, and San Diego, California USA".

RESULTS

Anti-inflammatory activity

Carrageenan induced paw oedema

The group treated with indomethacin showed maximum inhibition of oedema formation (92.03%). AW at all the doses (125, 250 and 500 mg/kg) showed significant inhibition of carrageenan induced paw oedema in rats in a dose dependent manner i.e.: 49.2, 64.6 and 87.7% respectively. The activity of AW at 500 mg/kg was almost equal to the inhibition comparable to standard (fig. 1).

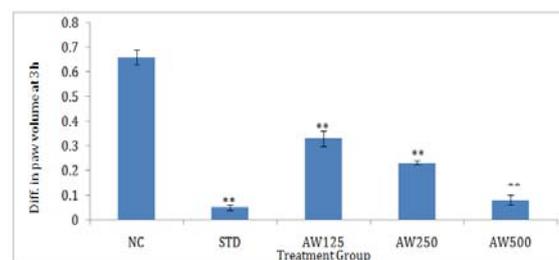


Fig. 1: Shows the effect of *Arenga wightii* ethanolic extract (AW)/Indomethacin on Carrageenan induced paw oedema in Wistar rats, Values are expressed as mean±SD, n = 6 (animals/group). **P ≤ 0.01 compared to normal control

Cotton pellet induced granuloma

AW at the three doses used in the study inhibited granulomatous tissue. The amount of exudates produced in response to insertion of cotton pellet was found to decrease dose dependently on treatment with AW (125, 250 mg/kg) extracts. The extract was potent in inhibiting both the exudative and proliferative phases of granuloma formation (table 1).

Table 1: Shows the effect of *Arenga wightii* ethanolic extract (AW)/indomethacin on granuloma tissue of cotton pellet-induced granuloma in rats

Treatment	Dose (mg/kg)	Granuloma Tissue	
		Wet wt.(mg)	Dry wt. (mg)
Pellet Control	-	0.335±0.07	0.075±0.013
Indomethacin	10	0.142±0.01** (57.6%)	0.037±0.004** (50.66%)
AW	125	0.22±0.02** (34.32%)	0.058±0.008** (22.66%)
AW	250	0.218±0.02** (34.92%)	0.054±0.016** (28%)
AW	500	0.192±0.02** (42.68%)	0.047±0.016** (37.33%)

Fig. in parentheses indicate % of inhibition of granuloma formation. (Values are mean±SD., n=6 (animals/group). ANOVA ** P≤0.01 vs. pellet control)

Acetic acid induced vascular permeability

AW at the three doses used in the study significantly inhibited acetic acid induced vascular permeability in mice.

AW at 125, 250 and 500 mg/kg showed 46.4, 72.83 and 78.21% of inhibitions while the standard drug, Indomethacin (10 mg/kg) produced 94.4% inhibition of vascular permeability (table 2).

Table 2: Shows the effect of *Arenga wightii* ethanolic extract (AW)/indomethacin on acetic acid induced vascular permeability

Treatment	Dose (mg/kg)	Absorption at 610 nm	% Inhibition
Normal Control	-	0.417	32.87
Toxin Control	-	0.219	
Indomethacin	10	0.022	94.47**
AW	125	0.128	46.43**
AW	250	0.059	72.83**
AW	500	0.055	74.84**

Values are the mean±SD, n=6 (animals/group). **P ≤ 0.05 compared to toxin control

Analgesic activity

Acetic acid induced writhing in mice

Intraperitoneal injection of acetic acid produced 50±1.54 writhes in the control group. AW produced a significant and dose dependent inhibition of writhes in Swiss albino mice. AW at 125, 250 and 500 mg/kg produced 47.3%, 52.6% and 69.3% inhibition of writhing respectively. The standard indomethacin produced 69.33% inhibition (fig. 2).

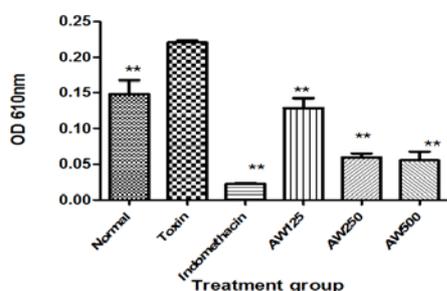


Fig. 2: Shows the effect of *Arenga wightii* (AW)/acetyl salicylic acid on acetic acid induced writhing response in mice. Values are the mean±SD n=6(animals/group). **P ≤ 0.01 compared to acetic acid control

Formalin induced paw licking

AW at the three doses 125, 250 and 500 mg/kg and sodium salicylate (100 mg/kg) showed the significant effect in both acute and delayed phases of the formalin test. AW at 125, 250 and 500

Table 3: Shows the effect of *Arenga wightii* (AW)/acetyl salicylic acid on formalin induced paw licking

Treatment	Dose (mg/kg)	Acute phase % inhibition	Delayed phase % inhibition
Normal Control	-	-	-
Sodium salicylate	100	85.26**	93.54**
AW	125	45.29	51.66
AW	250	66.60**	82.10**
AW	500	83.81**	91.69**

Values are the mean±SD, n=6 (animals/group). **P ≤ 0.01 compared to normal control

Cotton pellet induced granuloma is a model for chronic inflammation, in which the dry weights of the cotton pellets, represent the formation of granulomatous tissue [27, 28] which is caused by the proliferation of macrophages, neutrophils, fibroblasts, and multiplication of small blood vessels [29, 30]. Three phases of inflammatory response to subcutaneously implanted cotton pellet in rats have been described as (a) a transductive phase, that occurs during the first 3h, (b) an exudative phase occurring, between 3-72h after implantation of pellet and (c) a proliferative phase measured as increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation [31]. The suppression of the proliferation phase of sub-acute inflammation could result in the decrease in the weight of the granuloma formation [32]. It is presumed that AW was able to inhibit granuloma formation by suppressing or interfering with vital events like angiogenesis and/or other mediators.

mg/kg showed dose dependent reduction in pain (i.e.: 45.29%, 66.6% and 85.03%) which was similar to the standard (85.26%).

In the delayed phase sodium salicylate produced 98.43 % while the three doses of AW produced 95.67, 97.05 and 98.52% inhibition (table 3).

Toxicity study

In the toxicity study, no mortality occurred within 24 h with all the doses of AW tested. The LD₅₀ was therefore greater than 5000 mg/kg p. o, in mice (data not shown).

Preliminary phytochemical analysis

The preliminary phytochemical analysis of *Arenga wightii* revealed the presence of flavonoids, coumarins, tannins, cardiac glycosides, terpenoids, saponins and carbohydrates. TLC studies of AW revealed the presence of β-sitosterol and β-stigmasterol.

DISCUSSION

Inflammation is the local response of living mammalian tissues to injury. It is the body's defensive mechanism to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [25].

Carrageenan-induced paw edema is a standard and most commonly used technique to screen anti-inflammatory activity [16, 26]. It is expected that after tissue injury an animal will display spontaneous pain behaviour. This peripheral hypersensitivity or pain perception can be explained on the basis of local release of various inflammatory mediators i.e. bradykinin, prostaglandins or cytokines which can activate and sensitize the peripheral nerve endings.

Injection of acetic acid into the peritoneal cavity causes irritation leading to vasodilation and release of inflammatory mediators like histamine and nitric oxide which in turn leads to increased permeability of microvasculature that permits the escape of protein rich fluid in to the extra vascular compartment [33]. The ability of AW to reduce vascular permeability was reflected by the significant reduction in the dye leakage in a dose dependent manner. The anti-inflammatory activity of AW may be attributed to its activity to inhibit vascular permeability.

Acetic acid induced writhing is a sensitive procedure used to assess the activity of peripherally acting analgesics. Pretreatment with AW reduced the number of writhes induced by acetic acid in a dose dependent manner. These writhes may be related to the increase in peritoneal fluid level of PGE₂ and PGF_{2α} as postulated by Deraedt et

al., 1980[34]. Intraperitoneal administration of acetic acid also leads to increased levels of cyclooxygenase (COX) and lipooxygenase (LOX) products in peritoneal fluids as well as the release of many inflammatory mediators such as bradykinin, substance P, TNF- α , IL-1 α , IL-8, which eventually excites the primary afferent nociceptors entering dorsal horn of the central nervous system [35-37]. Therefore, the present study strongly suggests that the mechanism of AW may be linked partly to the inhibition of COX and/or LOX and other inflammatory mediators in peripheral tissues, there by interfering with the mechanism of signal transduction in primary afferent nociceptors.

The formalin test is a well described model of nociception and consists of two phases—early phase and late phase. The early phase is due to direct stimulation of the sensory nerve fibers by formalin which can be inhibited by centrally acting antinociceptives [20, 38], while the delayed phase response is due to mediators such as histamine, serotonin, prostaglandins and bradykinin [21, 39, 40].

Flavonoids have been reported to possess potential pharmacological activities such as anticancer, anti-ageing, antioxidant, anti-inflammatory, analgesic, etc. [41-43]. Apart from flavonoids, saponins and steroids are reported to possess anti-inflammatory and analgesic properties [44]. The anti-inflammatory and analgesic activity of *Arenga wightii* may be due to the presence of flavonoids, steroids, saponins etc. More study is in progress to decipher the exact mechanism responsible.

CONCLUSION

From the present study, it is concluded that *Arenga wightii* pith ethanolic extract has significant antinociceptive and anti-inflammatory activity and further studies are in progress to elucidate the exact mechanism of action.

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CONFLICT OF INTERESTS

Declared None

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