Vol. 9/2 (2009) 185 - 191

JOURNAL OF NATURAL REMEDIES

Effect of *Acacia* species on adjuvant-induced arthritis in rats

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Abstract

Acacia species (Mimosaceae) are used in Sudanese traditional medicine to treat various inflammatory diseases. The present study was designed to investigate the effect of dichloromethane (DCM) and 90% methanol extracts of bark and leaves of three *Acacia* species on cycoloxygenase-1 (COX-1) and -2 (COX-2) enzymes. The investigated species were: *A. nilotica* subsp. *tomentosa*, *A. nubica* and *A. senegal* subsp. *senegal*. The results showed that DCM bark extracts of all species showed high COX-2 selective inhibition (IC_{50} values of 0.45, 37 and 17.3 μg/mL) compared to COX-1 inhibition (IC_{50} values of 206.3, >250, >250 μg/mL) respectively. The DCM bark extracts of the three species were evaluated further *in vivo* in rats with adjuvant-induced arthritis. DCM bark extracts of *A. senegal* significantly reduced the edema when administered at a dose of 300 mg/kg. The extract did not cause lesions in the gastrointestinal mucosa compared to indomethacin which caused severe lesions in the small intestine (ulcer index = 194.3±2.7 mm^2). On the other hand, DCM bark extracts of *A. nubica* and *A. nilotica* were not active in *in vivo* assay. The results suggest that DCM bark extracts of *A. senegal* subsp. *senegal* may be useful in the treatment of rheumatoid arthritis.

Keywords: *Acacia*; Adjuvant arthritis; Anti-inflammatory activity; COX-1; COX-2; Mimosaceae.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease [1-2] that affects an estimated 0.5%-1% of the adult population world-wide [3]. RA is associated with a long-term loss of function and a significant socio-economic impact on individual sufferers and their families as well as the society as a whole [4]. The most effective medications for RA include COX-2 selective inhibitors and conventional nonselective non-steroidal anti-inflammatory drugs (NSAIDs). However, these drugs are associated with adverse effects such as gastrointestinal ulcers and thrombosis which resulted in the discontinued use of some of these drugs [5]. The genus *Acacia*

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(Mimosaceae) is widely distributed in the arid and semi-arid belt of Sudan. Species within the genus have many ethnomedicinal uses across the country [6]. Powdered leaves and bark of *A. nilotica*, are used externally to treat eye inflammation while a decoction prepared from the bark is used to treat fevers. A poultice made from the powdered leaves of *A. nubica* is used to treat swelling [7] while different parts of *A. senegal* are used for the treatment of genito-urinary inflammation and cold [6]. Adjuvant induced arthritis is a model of chronic inflammation that exhibits several pathological changes similar to those occurring in RA [2]. Hence, the present study was undertaken to evaluate three *Acacia* species, namely *A. nilotica* subsp. *tomentosa*, *A. nubica* and *A. senegal* subsp. *senegal* for the treatment of adjuvant induced arthritis in rats and *in vitro* COX inhibitory activity as part of a program to find new agents for the treatment of RA with reduced adverse effects.

2. Materials and methods

2.1 Plant material and extraction

Bark and leaf material of *Acacia* species were collected from the Kenana area, Sudan. The identity of the plants was confirmed by Dr. Esameldin Warrag, Faculty of Forestry, University of Khartoum, Sudan. Voucher specimens were deposited in the herbarium of The Forest Research Centre, Soba, Sudan.

For *in vitro* COX assays, dried and powdered barks and leaves (1 g) were extracted sequentially with 10 µL dichloromethane and 90% methanol in an ultrasound bath for one hour. Extracts were filtered and evaporated to dryness.

For *in vivo* assay, bark material of *A. nilotica* (450 g), *A. nubica* (500g) and *A. Senegal* (1 kg) were extracted with 2 L of dichloromethane for 24 h, three times each to give 5.23, 6.5 and 11 g of crude extracts, respectively.

2.2 Cyclooxygenase (COX) assays

COX-inhibitory activity was determined using the COX-1 and COX-2 assays [8-9]. Briefly, 10 µL of COX-1 or COX-2 enzyme (3.0 units; Sigma Chemical Company, St Louis, MO) were activated with 50 µL co-factor solution (0.9 mM L-epinephrine, 0.49 mM glutathione and 1 µM hematin in 0.1 M Tris buffer, pH 8.0) on ice for 5 min. Both the enzyme solution (60 µL) and sample solution (2.5 µL ethanolic plant extract applied to 17.5 µL water) were incubated at room temperature for 5 min. The reaction was started by adding 20 µL [1-^{14}C]arachidonic acid (30 µM, 17 Ci/mol). Samples were incubated for 10 min at 37°C and the reaction was terminated by adding 10 µL 2 M HCl. The unmetabolized arachidonic acid was separated from the prostaglandin products by column chromatography. The percentage inhibition of the test solutions was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank (2.5 µL ethanol and 17.5 µL water). Positive control measurements were carried out with indomethacin. The results given are the mean ± S.E.M. of four experiments in duplicate (% inhibition).

2.3 In vivo screening

2.3.1 Animals

Experiments were performed using male Wistar rats (6 weeks old, 180-220 g) bred and raised in the animal house of the Faculty of Agriculture, Tottori University. The animals were maintained and the experiments were performed according to the regulations of Tottori University, Tottori, Japan. A minimum of six animals were used in each group.

2.3.2 Adjuvent-induced hind paw edema model [10]

Adjuvant arthritis was induced in the right hind paw by subcutaneous injection of 50 µL of heat-killed *M. tuberculosis* suspended in liquid paraffin

(10 mg/mL). Initially, the volumes of the right and left hind paws were measured immediately before the injection of the adjuvant and 15 and 16 days later by the method of water displacement. An oral dose of either the DCM bark extracts of *A. nilotica* (300 mg/kg), *A. nubica* (300 mg/kg) and *A. senegal* (300 and 100 mg/kg) were given on day 15 to the treatment groups. Indomethacin (10 mg/kg) was used as the standard drug, while animals in the control group received 2 ml/kg of 70% ethanol orally as a vehicle (which was used to reconstitute the crude extracts).

In a separate experiment, the DCM extracts of *A. senegal* (300 and 100 mg/kg) and *A. nilotica* (300 mg), together with indomethacin and the control, were administered orally once a day for three days beginning on day 15 after the injection of the adjuvant. The volumes of the right and left hind paws measured immediately before injection and on days 15, 16, 17 and 18 of the experiment using the water displacement method. The animals were sacrificed by ether overdose on day 18. The stomachs were removed and washed with 1% formalin solution. The small intestines were opened along the anti-mesenteric attachment and the contents removed. The lesions in the stomach and small intestine were measured under a dissecting microscope with a 1-mm square-grid eyepiece (x 10) [11-13]. The area of visible lesions was measured, and the ulcer index was expressed as the sum total area in mm^2 of individual lesions.

2.4 Statistical analysis

All data are expressed as mean ± S.E.M. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Results were considered significant at $p < 0.05$.

3. Results

3.1 In vitro assay

Results of the COX-inhibitory activity of crude extracts (250 μg/mL) of both leaves and bark of *Acacia* species are summarized in Table 1. DCM extracts, in general, showed high inhibitory activity in both COX-1 and -2 assays compared to the 90% MeOH extracts, except in the case of leaf extracts of *A. nilotica* and *A. nubica*. All DCM extracts inhibited COX-2

Table 1. Percentage prostaglandin synthesis inhibition detected in dichloromethane (DCM) and 90% methanolic (MeOH) extracts from some *Acacia* species in Sudan using the COX-1 and COX-2 assays (at 250 μg/mL).

Plant species	Plant part	COX-1		COX-2	
		DCM Extract	MeOH Extract	DCM Extract	MeOH Extract
A. nilotica (L.) Willd. ex Del. subsp. *tomentosa*	Bark	46.3 ± 6.7	50.9 ± 2.4	83.9 ± 4.2	44.1 ± 3.4
	Leaf	78.1 ± 0.3	98.8 ± 0.20	89.5 ± 1.8	85.5 ± 3.2
A. nubica Benth.	Bark	39.6 ± 5	0.8 ± 0.3	90.4 ± 4.6	28.3 ± 0.9
	Leaf	77.4 ± 4.6	89.4 ± 7.1	92.7 ± 0.2	72.5 ± 1.2
A. senegal (L.) Willd. Subsp. *senegal*	Bark	17.5 ± 7.1	19.2 ± 3.3	91.6 ± 5.9	NA
	Leaf	71.9 ± 7.43.1	43.8 ± 7.8	93.7 ± 3.4	92.7 ± 8.7
Indomethacin (IC_{50} in μM)		2.1±0.22		131.7±6.5	

Values are mean ± S.E.M; n=4. [a]NA: not active

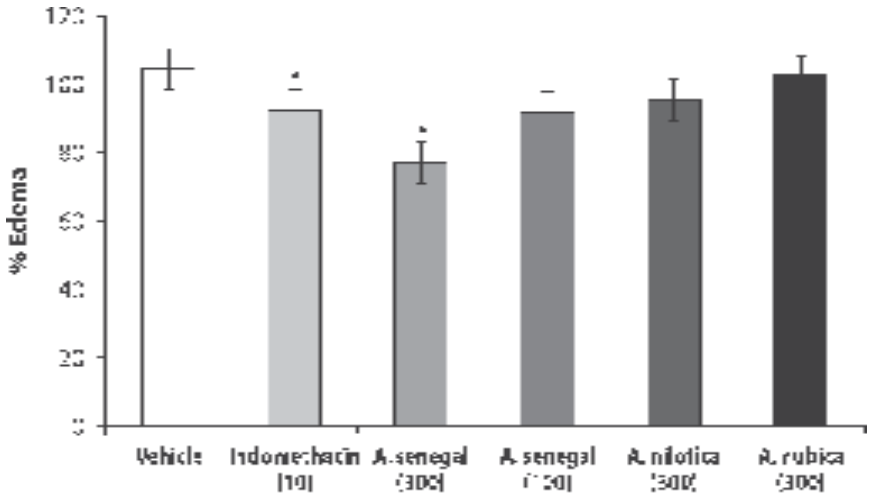

Fig. 1. Effect of DCM extracts of *Acacia* species on paw edema in rats with adjuvant arthritis. Each column and bar represents the mean value ± S.E.M of 6 rats. The doses of 300 mg/kg and 100 mg/kg of the extracts of *A. senegal* and only the dose of 300 mg/kg for both *A. nilotica* and *A. nubica* were administered orally. Indomethacin was administered at a dose of 10mg/kg. *p<0.05 compared to the vehicle.

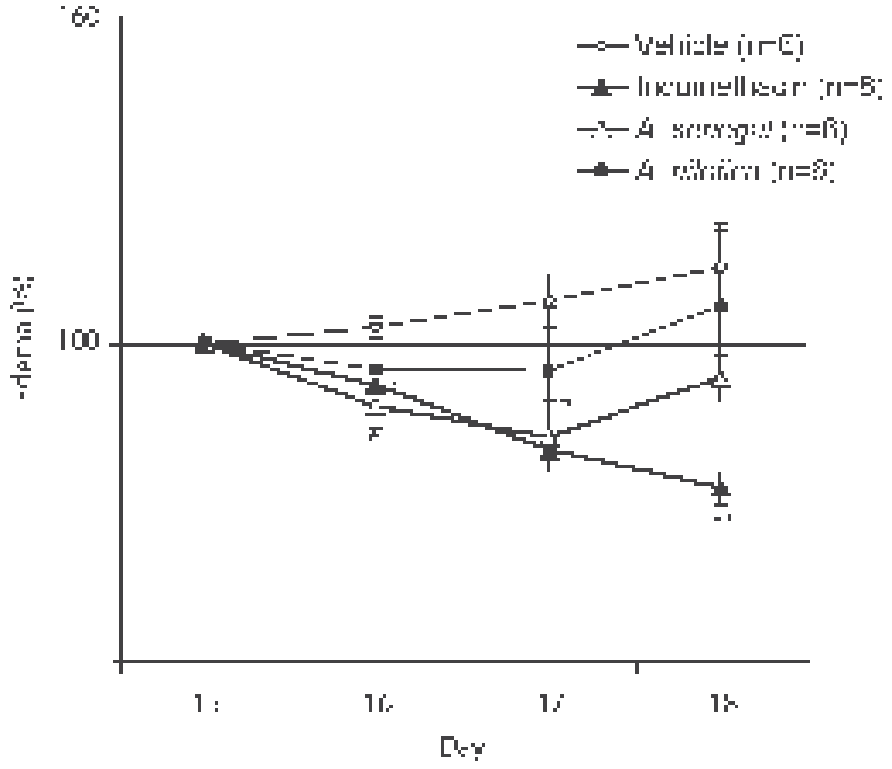

Fig. 2. Effects of *A. nilotica* and *A. senegal* DCM extract (300 mg/kg/day) on edema in rats with adjuvant arthritis. Each line and bar represents the mean value ± S.E.M of 6-8 rats. The extracts were given orally at a dose of 300 mg/kg/day. Indomethacin was administered at a dose of 10mg/kg/day. * p<0.05, while **p<0.001 as compared to the vehicle.

catalysed prostaglandin biosynthesis by as much as 83 - 94% while 90% MeOH extracts from the leaves were more active (72 – 92%) than the bark extracts (50% or less).

IC_{50} values were determined for extracts that showed high COX-2 selective inhibition. These were DCM bark extracts of *A. nubica*, *A. nilotica* and *A. senegal* where IC_{50} values against COX-2 were 37, 0.45 and 17.3 µg/ml respectively, compared to COX-1 inhibition (IC_{50} values were >250, 206 and >250 µg/mL). Positive control measurements were carried out with indomethacin (IC_{50} values were 2.1 µM for COX-1 and 131.7 µM for COX-2).

3.2 In vivo assay

Results obtained from *in vivo* experiments where extracts were administered for one day are shown in Figure 1. Only DCM extracts of *A. senegal*, at the highest dose (300 mg/kg), significantly reduced the swelling induced by the adjuvant in the right hind paw of the rats. The lowest dose (100 mg/kg) of the *A. senegal* extract and *A. nilotica* extract (300 mg/kg) produced a slightly non-significant effect while *A. nubica* extracts (300 mg/kg) had no effect.

Results of the anti-inflammatory effect of DCM bark extracts of *A. nilotica* and *A. senegal* administered to rats with adjuvant-induced arthritis for three consecutive days are shown in Figure 2. DCM bark extracts of *A. senegal*, at a dose of 300 mg/kg, significantly reduced the swelling induced in the right hind paw of the rats. The extract resulted in a further reduction in edema by the second day although a decrease in the inhibitory activity was observed on day three. There was either a slight decrease or a considerable paw volume increase in the groups where DCM bark extracts of *A. nilotica* and the vehicle were administered. Indomethacin caused a significant reduction in the swelling of the hind paw of arthritic rats at a dose of 10 mg/kg. Oral administration of indomethacin (10 mg/kg) induced many severe lesions in the small intestine within three days, the ulcer index being 194.3 ± 2.7 mm^2. Neither the active DCM extract of *A. senegal* nor the inactive extracts of both *A. nilotica* and *A. nubica* induced any lesions in the small intestine during the test period.

4. Discussion

Preliminary screening of the DCM and 90% MeOH extracts of the bark and leaves of the different *Acacia* species revealed that the DCM extracts of the barks of *A. nilotica*, *A. nubica* and *A. senegal* had high COX-2 selective inhibition and that the DCM bark extracts of *A. nilotica* were the most active. However, where extracts were administered for one day in *in vivo* experiments, only extracts of *A. senegal* significantly reduced the swelling of the rat paw edema and in a dose dependent manner (Fig. 1). DCM extracts of *A. senegal* bark also significantly reduced the edema in chronic adjuvant-induced arthritic rats when the extracts were administered for three consecutive days.

Adjuvant-induced arthritis in rats is the most widely used model of experimental arthritis in screening programs for anti-inflammatory drugs [10]. Inflammation is a complex process in which many different mediators are involved including kinins, platelet activating factors, prostaglandins and leukotriens [14]. However, the results from the COX assays suggest that the mechanism of action for the *A. senegal* crude extracts was mediated through the inhibition of COX-enzymes.

Among the plants investigated in this study, the only plant species that has been investigated with respect to anti-inflammatory activity is *A. nilotica*. The androstene steroid isolated from this species showed activity against TPA-induced mouse ear edema [15]. Extracts from

Acacia nilotica were, also, reported to have an inhibitory effect on Hepatitis C virus protease [16] and HIV-1 protease [17] as well as antiplasmodial [18] and molluscicidal effects [19]. Interestingly, *A. nilotica* extracts had no effect on the edema in rats both in the short and long-term when administered orally or subcutaneously (data not shown). *Acacia* species, including *A. nilotica* and *A. nubica*, contain tannins, ethyl galate and flavonoids. These compounds, and the tannins in particular, are responsible for the majority of false positive anti-inflammatory activities observed [20-23]. Difficulties in absorption could also explain the negative effects of crude extracts of *A. nilotica in vivo*.

Gastrointestinal ulcers and lesions are produced by most NSAIDs, to varying degrees, most probably by inhibiting the synthesis of gastro-protective prostaglandins [24]. The inflammatory mediators - leukotrienes, platelet activating factors and intracellular calcium - have been implicated in the development of the gastrointestinal lesions induced by NSAIDs [12].

This study revealed that DCM crude extracts of *A. senegal* are effective anti-inflammatory inhibitors which, unlike indomethacin, did not cause gastrointestinal ulcers or lesions. Many plant extracts are known to contain anti-ulcer agents [25-26] and leukotriene-biosynthesis inhibitors [27]. However, the fact that the crude extracts did not cause ulcers, may be due to the safety of the active anti-inflammatory constituent(s) or, alternatively, to the presence of either anti-ulcer, leukotriene-biosynthesis inhibitors, platelet activating factors or intracellular calcium antagonists in the crude extracts. This aspect requires further investigation.

In conclusion, the present results revealed that extracts of *A. senegal* represent a good source of anti-inflammatory principles and that this *in vivo* anti-inflammatory activity is mediated by prostaglandin synthesizing COX enzymes. The study also raises some concern about reports that identify activity in crude extracts in *in vitro* studies only, without comparison to activity in *in vivo* assays. The next step is to isolate and characterize the active constituent(s) and to establish its effect on platelet aggregation and cardiovascular diseases.

5. Acknowledgements

This research was supported by a fellowship from the Japanese Society for the Promotion of Science (JSPS), Tokyo, Japan.

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