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

Tsueirola F. Uldjassiri 1, Naoki Wada 1, Yoshimasa I. Wajrak 1, Hiroshi Satoh 1

1. Department of Veterinary Pharmacology, Faculty of Agriculture, Tohoku University, Aizuwakamatsu, Japan.
2. Department of Silviculture, Faculty of Forestry, University of Khartoum, Shendi, Sudan.

**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 as *A. cyclops*, *A. COX-1* 0.2 g and 0.3 g (COX-2) mg/kg. The investigated species were: *A. nilotica* (DCM extract), *A. nilotica* (DCM extract), and *A. nilotica* (methanol extract). The results showed that DCM bark extracts of all species showed high COX-2 selective inhibitors (6 g/kg of p.o. 0.45, 0.37 and 17.3 μg/mL) compared to COX-2 inhibitors (6 g/kg of p.o. 0.45, 0.37 and 17.3 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 0.95 g/kg of p.o. reduced the edema in a dose of 0.3 mg/kg. The extract did not cause toxicity in the experimental animals. The results suggest that the bark extract of *A. nilotica* 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 that affects an estimated 0.5%–1% of the adult population worldwide [1]. 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 [2]. The most effective medications for RA include COX-2 selective inhibition 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 [3]. The genus *Acacia*...

(Mniscesus) is widely distributed in the arid and semi-arid belt of Sudan. Species within the genus have many ethnopharmacological 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. saundersii 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. somaliensis, A. nilotica and A. senegal subsp. senegal, for the treatment of adjuvant induced arthritis in rats and in vivo COX inhibition 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 Kerinci area, Sumatra. The identity of the plants was confirmed by Dr. Eusteadin Warrag, Faculty of Forestry, University of Khartoum, Khartoum, Sudan. Specimens were deposited in the herbarium of The Forest Research Centre, Khartoum, Sudan.

 For in vivo COX assays, dried and powdered bark's (1 g) were extracted sequentially with 10 mL 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. nilotica (900 g) and A. senegal (1 kg) were extracted with 2 L of dichloromethane for 24 h, three times each to give 5.25, 6.5 and 11 g of crude extracts, respectively.

2.2 Cyclooxygenase (COX) assay

 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 (2.0 μL; Sigma Chemical Company, St Louis, MO) were activated with 50 μL co-factor solution (0.9 mM L-epinephrine, 0.45 μM glutathione and 1 μM NEM in 0.1 M Tris buffer, pH 8.0) on ice for 5 min. Both the standard 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 [1-14C] arachidonic acid (30 μM, 175 mCi). 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 (5% 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, Tokai University. The animals were maintained and the experiments were performed according to the regulations of Tokai University, Ibaraki, Japan. A minimum of six animals were used in each group.

2.3.2 Adjuvant-induced hind paw oedema model

 Adjuvant arthritis was induced in the right hind paw by subcutaneous injection of 50 μL of heat-killed M. bovis bactferment suspended in liquid paraffin
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. nubicata* (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 after 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 stumps 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 (×10) [11-13]. The area of visible lesions was measured, and the ulcer index was expressed as the sum total area in mm² of individual lesion.

### 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. nubicata*. All DCM extracts inhibited COX-2

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant part</th>
<th>COX-1</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. nilotica</em> (L.)</td>
<td>Bark</td>
<td>46.3 ± 6.7</td>
<td>50.9 ± 2.4</td>
</tr>
<tr>
<td>Wild ex. Del. subsp. sommaria</td>
<td>Leaf</td>
<td>78.1 ± 0.3</td>
<td>98.8 ± 0.9</td>
</tr>
<tr>
<td><em>A. nubicata</em> Brook.</td>
<td>Bark</td>
<td>39.6 ± 5</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Wild Subsp. senegal</td>
<td>Leaf</td>
<td>77.4 ± 4.6</td>
<td>99.4 ± 7.1</td>
</tr>
<tr>
<td><em>A. senegal</em> (L.)</td>
<td>Bark</td>
<td>17.5 ± 7.1</td>
<td>19.2 ± 3.3</td>
</tr>
<tr>
<td>Wild Subsp. senegal</td>
<td>Leaf</td>
<td>71.5 ± 4.31</td>
<td>43.8 ± 7.8</td>
</tr>
</tbody>
</table>

Indomethacin (IC₅₀ in μM)

- COX-1: 2.1±0.22
- COX-2: 131.7±6.5

Values are mean ± S.E.M., n=4. NA: not done.
Fig. 1. Effect of DCM extracts of *A. semeig* on paw edema in mice with adjuvant arthritis. Each column and bar represents the mean value ± S.E.M of 3 rats. The doses of 300 mg/kg and 100 mg/kg of the extracts of *A. semeig* and only the dose of 300 mg/kg for butanol, salsola and *A. nubica* were administered orally. Indomethacin was administered at a dose of 10 mg/kg. *p<0.05 compared to the vehicle.

Fig. 2. Effect of *A. salsola* and *A. semeig* DCM extract (100 mg/kg/die) on paw edema in mice with adjuvant arthritis. Each line and bar represents the mean value ± S.E.M of 4 rats. The extracts were given orally at a dose of 300 mg/kg/day. Indomethacin was administered at a dose of 10 mg/kg/day. *p<0.05, while **p<0.01 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. nubic, A. nilotic 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. nilotic 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. nilotic 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. nilotic and the vehicle were administered. Indomethacin caused a significant reduction in the swelling of the hind paw of arthritis 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 198.4 ± 2.7 mm². Neither the active DCM extract of A. senegal nor the inactive extracts of both A. nilotic 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. nilotic, A. nubica and A. senegal had high COX-2 selective inhibition and that the DCM bark extracts of A. nilotic were the most active. However, where extracts were administered for one day in vivo experiments, only extracts of A. senegal significantly reduced the swelling of the rat paw edema in a dose dependent manner (Fig. 1). DCM extracts of A. senegal bark also significantly reduced the edema in chronic adjuvant-induced arthritis rats when the extracts were administered for three consecutive days. Adjuvant-induced arthritis rat is the most widely used model of experimental arthritis in screening program for anti-inflammatory drugs [10]. Inflammation is a complex process in which many different mediators are involved including kinins, platelet activating factors, prostaglandins and leukotrienes [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. nilotic. The endogenous 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 H1N1 protease [17] as well as antiproliferative [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. nilica, 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 synthetase-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.

References