http://dx.doi.org/10.4314/jpb.v8i2.3
Vol. 8 no. 2, pp. 80-85 (September 2011)
http://ajol.info/index.php/jpb

Journal of
**PHARMACY AND BIORESOURCES**

# Effects of standardized methanol extract of *Andrographis paniculata* on guinea pig tracheal cyclooxygenase and rat paw inflammation

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Received 1st June 2011; Accepted 4th July 2011

**Abstract**
Extracts of *Andrographis paniculata* Nees is used in traditional medicine for the treatment several diseases including inflammation, asthma and common cold. The aim of this study was to investigate the possible effects of a clinical dose of the methanol extract of *A. paniculata* (AP) on tracheal cyclooxygenase and inflammation in animals. Ovalbumin (OA) sensitized guinea pigs were orally treated daily for 21 consecutive days with water, 2.86 mg/kg AP, or 10 mg/kg prednisolone. At the end of treatment tracheae were excised and assayed for cyclooxygenase enzyme. The same dose of AP was tested on acute inflammation in rat using 10 mg/kg indomethacin as the positive control. Although 2.86 mg/kg of AP did not significantly reduce guinea pig tracheal cyclooxygenase activity when compared with control it significantly ($P < 0.05$) reduced rat paw size within the first hour of induction of inflammation. The clinical dose of AP used for the study possesses anti-inflammatory property but higher doses may be more effective in the inhibition of airway cyclooxygenase.

*Keywords*: *Andrographis paniculata*; Methanol extract; Asthma; Inflammation; Cyclooxygenase

## INTRODUCTION

Herbal remedies for the management of airway diseases are becoming popular owing to their cheapness, availability and safety profile when compared to orthodox medicines. Such herbal remedies include the extracts of *Andrographis paniculata* Nees (Acanthaceae). This plant is widely distributed in Asia. It seems to have very recently been introduced to other parts of the world including Nigeria (Obodozie *et al.*, 2010). The plant grows to a height of 30-110 cm in moist shady places. It has glabrous leaves and produces white flowers with rose-purple spots on the petals. The stem is dark green, quadrangular with longitudinal furrows and wings on the angles of the younger parts (Jarukamjorn and Nemeto, 2008).

The plant has been used since ancient times in traditional medicine in Asia (Jarukamjorn and Nemeto, 2008). Various pharmacological effects have been associated with the extracts and the constituents. These include: antimalarial (Rahman *et al.*, 1999; Mishra *et al.*, 2009), antiinflammatory (Sheeja *et al.*, 2006; Abu-Ghefreh *et al.*,

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ISSN 0189-8442

2009), anti-diabetic (Wibudi *et al.*, 2008; Dandu and Inamdar, 2009), and antitumour (Kumar *et al.*, 2004; Zhao *et al.*, 2008). Other properties include: hepatoprotective (Kapil *et al.*, 1993; Trivedi and Rawal, 2001), analgesic (Madav *et al.*, 1995), vasorelaxant and cardioprotective (Yoopan et al., 2007; Woo *et al.*, 2008), uterine relaxant (Burgos *et al.*, 2001). The extracts and its constituents have also shown antiviral (Wiart *et al.*, 2005; Lin *et al.*, 2001), antidiarrhoeal (Gupta *et al.*, 1990) and antibacterial (Singha *et al.*, 2003) properties. We have demonstrated the relaxant effect of the aqueous leaf extract on airway smooth muscles (Ozolua *et al.*, 2009). The standardized methanol extract of the plant is marketed as KalmCold® by Natural Remedies Pvt. Ltd. (Bangalore, India) for the treatment of common cold. This preparation is exported to other parts of the world.

Andrographolide and its closely related diterpines such as neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide are the main constituents of the plant and are believed to be responsible for its pharmacological actions (Hu and Zhou, 1982; Jarukamjorn and Nemeto, 2008). There seems to be consistency in the HPLC-DAD-MS fingerprints of aerial parts of the plant obtained from different regions of the world (Arpini *et al.*, 2008).

Inflammation is an accompaniment of chronic obstructive airway diseases such as asthma (Murdoch and Lloyd, 2010) and cyclooxygenase enzyme is a mediator of inflammatory response (Nagai, 2008). Effectiveness in treatment of common cold, ability to relax airway smooth muscles and inhibition of inflammation are closely related useful properties of the plant for the management of obstructive airway diseases. We therefore designed this study to investigate if the clinical dose for the treatment of common cold would also inhibit tracheal cyclooxygenase and inflammation using animal models.

## EXPERIMENTAL

**Extract.** Standardized dried methanol extract of *A. paniculata* manufactured by Natural Remedies Pvt. Ltd. (Bangalore, India) was used for the study. The label characteristics include: batch number – RD/2114, date of manufacture – November 2008, colour – green to brownish green powder, taste – bitter, and bulk density – 0.20 to 0.80. Other characteristics include: moisture – 5.0% and active constituent – andrographolide (30%). It was stored in the original company sachets (airtight) at 4$^{o}$C. The extract was reconstituted in distilled water before administration to the animals.

**Animals.** Guinea pigs of either sex (300-400 g) and Wistar rats of either sex (220-250 g) were used for the experiments. The guinea pigs were procured from the Animal House, Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. The rats were obtained from the Animal House Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. All animals were allowed free access to pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and tap water. They were exposed to natural lighting condition and room temperature. They were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA, 2002). The study was approved by institutional ethical committee on the use of animals for experiments.

**Tracheal cyclooxygenase assay.** Guinea pigs were randomly allotted into three groups comprising of:

1. Ovalbumin (OA) sensitized + distilled water treated. These guinea pigs were sensitized by intraperitoneal injection of 100 mg OA, another intramuscular injection of 50 mg OA and a booster dose of 50 mg OA (intramuscular) 24 h later (Bramley *et al.*, 1995).

2. OA sensitized + 2.86 mg/kg/day AP orally. The animals in this group were sensitized but treated with AP for 21 consecutive days. The dose of AP was obtained by dividing the clinical daily dose of 200 mg by the physiological human weight of 70 kg.
3. OA sensitized + 10 mg/kg/day prednisolone (PRED) orally. The animals were sensitized but treated with prednisolone for 21 consecutive days.

One hour after the 21$^{st}$ day dose, the guinea pigs were sacrificed and tracheae were excised and placed in Petri dishes containing ice-cold distilled water. The tracheae were each weighed and homogenized using a mortar containing 1 g of acid-washed sand and 5 ml of ice-cold water. The homogenates were transferred to plain bottles and centrifuged at 3000 rpm for 20 min. The supernatants were stored in a deep freezer at -20$^{o}$C. Tracheal cyclooxygenase assay was carried out colorimetrically at 590 nm (Kulmacz and Lands, 1983).

**Evaluation of anti-inflammatory activity.** The method used has been previously described (Ching *et al.*, 2009). Rats were randomly allotted to three groups of five animals per group. The animals were fasted overnight but with free access to water which was only withdrawn during the experiment. The animals were orally administered normal saline (5 ml/kg), AP (2.86 mg/kg) or indomethacin (10 mg/kg). One hour later carrageenan (0.1 ml of 1 % w/v in normal saline) was injected into the right hind paw of each rat. Measurement of the circumference of the injected paw was done by use of Vernier caliper at hourly intervals for a maximum of four hours after carrageenan injection. Paw swelling at each time point was taken as the difference between the paw diameter at the specific time point and that before the injection of carrageenan.

**Drugs and chemicals.** Indomethacin was a free donation by Chemech (now Chemiron) Pharmaceutical Industries Ltd (Nigeria). Carrageenan was manufactured by Sigma (UK) while cyclooxygenase kit was supplied by Cayman Chemical Company (Ann Arbor, USA). Drugs were prepared fresh each day before administration.

**Statistical analysis.** All data are expressed as Mean ± standard error of mean (SEM). Comparison between controls and the test groups was done using Student's t-test. $P < 0.05$ was considered significant.

## RESULTS

Daily treatment of sensitized guinea pigs with 2.86 mg/kg of AP for 21 consecutive days did not significantly inhibit tracheal cyclooxygenase enzyme activity compared to control. On the other hand, daily administration of 10 mg/kg of prednisolone significantly ($P < 0.05$) reduced tracheal activity of the enzyme (Figure 1). The values (units/g tissues) are: OA – 59.8 ± 11.5; AP – 32.4 ± 5.4 and PRED – 28.2 ± 5.7.

Table 1 shows that swelling increased in the paw of control rats within the first hour after injection of carrageenan. Compared to saline control within the first hour, AP significantly ($P < 0.01$) reduced paw oedema but not in subsequent hours. The inhibitory effect of indomethacin was consistent and significant ($P < 0.005$) throughout the 4 hours of monitoring.

## DISCUSSION

The present data have shown that the clinical dose of AP that is used in the treatment of common cold did not significantly inhibit tracheal cyclooxygenase enzyme in sensitized guinea pigs. However in rats, the dose was able to inhibit paw inflammation within the first one hour. Previous reports have demonstrated the anti-inflammatory effects of extracts of AP

(Sheeja *et al.*, 2006; Abu-Ghefreh *et al.*, 2009).

The mechanism of this action may be associated with the inhibition of pro-inflammatory mediators (Chandrasekaran *et al.*, 2010). Significant inhibition of $PGE_2$ production in murine macrophages was observed at AP concentration of 50 µg/ml (Chandrasekaran *et al.*, 2010). Assuming 100% bioavailability, we have estimated that the dose in the present study would be equivalent to 35.8 µg/ml blood concentration for a 400 g guinea pig using Anchill's (1956) estimation of guinea pig blood volume. Thus the dose may have been too low for significant cyclooxygenase inhibition in the guinea pig tracheae. AP inhibits other inflammatory mediators such as nitric oxide and interleukins (Chandrasekaran *et al.*, 2010).

The guinea pigs in the present study were sensitized and pretreated with the extract. Sensitization with ovalbumin is a model of obstructive airway disease in which the airways become inflamed and hyper-responsive (Shin *et al.*, 2009; Ozolua *et al.*, 2009). Ability of agents to inhibit responses by ovalbumin-sensitized guinea pigs often suggests usefulness in obstructive airway diseases. Similarly, carrageenan rat paw oedema model is used to assess the ability of agents to inhibit acute inflammation (Ching *et al.*, 2009).

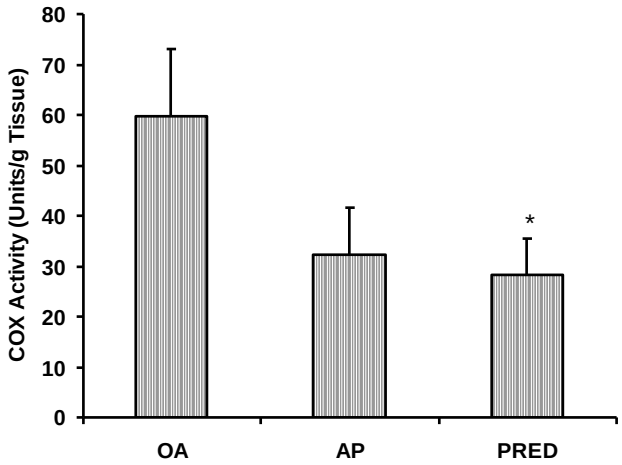


**Figure 1.** Total cyclooxygenase (COX) activity in ovalbumin sensitized (OA), sensitized but 2.86 mg/kg/day x 21 days methanol extract *A. paniculata* treated (AP), and sensitized but 10 mg/kg/day x 21 days prednisolone (PRED) treated guinea pigs. * $P < 0.05$ compared to control. n = 5 per group.

**Table 1.** Effect of methanol extract of *A. paniculata* (AP) on carrageenan-induced rat paw oedema

| Group | Paw circumference (mm) | | | | |
|---|---|---|---|---|---|
| | 0 h | 1 h | 2 h | 3 h | 4 h |
| Saline (5 ml/kg) | 4.98 ± 0.05 | 7.42 ± 0.20 | 6.74 ± 0.16 | 6.86 ± 0.20 | 6.88 ± 0.20 |
| AP (2.86 mg/kg) | 5.86 ± 0.24 | 6.72 ± 0.09* | 6.48 ± 0.13 | 6.40 ± 0.08 | 6.38 ± 0.01 |
| Indomethacin (10 mg/kg) | 4.54 ± 0.12 | 6.18 ± 0.25** | 5.78 ± 0.21** | 5.52 ± 0.12** | 5.34 ± 0.06** |

*$P < 0.01$, **$P < 0.005$ compared to corresponding saline value at the time point. n = 5 per group.

In spite of the results from the present study, it seems likely that higher doses would inhibit the enzyme significantly and demonstrate better anti-inflammatory activity. The use of such higher doses for clinical situations may be supported by the fact that the extract has been found to be relatively adverse effects free. In rodents, a high dose of 5000 mg/kg has not been associated with any adverse effects (Chandrasekaran *et al.*, 2009).

Chronic airway obstructive diseases such as asthma involve inflammation and airway smooth muscle hyper-responsiveness (Lemanske *et al.*, 2010; Murdoch and Lloyd, 2010). These diseases are often worsened by acute common cold (Johnston *et al.*, 1995; Guilbert and Denlinger, 2010). A previous study in our laboratory has shown that the aqueous extract of AP prevents tracheal smooth muscle responses to spasmogens (Ozolua *et al.*, 2009). Taken together with anti-inflammatory (Sheeja *et al.*, 2006; Abu-Ghefreh *et al.*, 2009; Chandrasekaran *et al.*, 2010) and antiviral (Wiart *et al.*, 2005; Lin *et al.*, 2001) properties, AP is a candidate for airway diseases.

In conclusion the clinical dose of AP used in this study has shown anti-inflammatory property that may be helpful in obstructive airway disorders that involve inflammation but higher clinical doses may be more useful.

**Acknowledgments**

The authors are grateful to the technical staff of the Department of Biochemistry and Department of Chemical Pathology, University of Benin for their assistance. Natural Remedies Pvt. Ltd. (India) kindly donated KalmCold® and the test kit for cyclooxygenase. The study was a final year project dissertation by ACO.

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