

# PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS

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## A Drug Used in Traditional Medicine, *Harpagophytum procumbens*: No Evidence for NSAID-like Effect on Whole Blood Eicosanoid Production in Human

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**ABSTRACT.** Devil's Claw (*Harpagophytum procumbens*), an herbal product being marketed in Canada and in Europe as a home remedy for the relief of arthritic disease, was investigated in healthy humans on eicosanoid production during spontaneously blood clotting. Volunteers took *H. procumbens* (daily 4 capsules of 500 mg powder containing 3% of total glucoiridoids) for a period of 21 days. The following are the results (mean (SEM)): before *H. procumbens* intake, prostaglandin (PG)E<sub>2</sub> (ng/ml serum): 2.1 (0.4) (n = 25), thromboxane (TX)B<sub>2</sub>: 147 (27) (n = 25), 6-keto-PGF<sub>1α</sub>: 4.4 (0.7) (n = 13), leukotriene (LT)B<sub>4</sub>: 3.4 (0.4) (n = 25); after intake: PGE<sub>2</sub>: 3.2 (0.6), TXB<sub>2</sub>: 143 (24), 6-keto-PGF<sub>1α</sub>: 4.2 (0.9), LTB<sub>4</sub>: 3.8 (0.6). Each subject serving as her own control, no statistically significant differences were observed between before and after *H. procumbens* intake. These results indicate that Devil's Claw lacks, at least in healthy humans and under the selected conditions, the biochemical effects on arachidonic acid metabolism of antiarthritic drugs of the non-steroidal antiinflammatory type.

### INTRODUCTION

*Harpagophytum procumbens* DC. (Pedaliaceae), or Devil's Claw, a herbaceous plant originating from South Africa, is being marketed like preparations from the secondary lateral roots in Canada and in European (especially in France, Germany and Great Britain) pharmacy and herborist's shop as a home remedy for the treatment of various inflammatory diseases, including rheumatoid arthritis (1). Major active components of *H. procumbens* are thought to be three iridoid glucosides (harpagide, harpagoside and procumbide). Views concerning the safety and efficacy of *H. procumbens* have generally been based upon folklore and testimonials, but predicated at best on very limited scientific information (2–6). Particularly, the mechanism by which *H. procumbens* medicinal properties could be accounted for is not known.

Prostanoids, cyclooxygenase products of arachidonic acid, belong to the mediators of acute inflammation and they are also suggested to be involved in the pathogenesis of rheumatoid arthritis (7, 8). On the other

hand, prostanoids (mainly prostaglandin (PG)E<sub>2</sub>) are been reported to have antiinflammatory properties in some forms of experimental inflammation (7, 9). Furthermore, leukotrienes (mainly LTB<sub>4</sub>), 5-lipoxygenase products of arachidonic acid, are also suggested to act as mediators in inflammation diseases such as rheumatoid arthritis (10). The therapeutic action of non-steroidal antiinflammatory drugs (NSAIDs) and that of anti-inflammatory steroids can partly be explained by their inhibitory effects on prostanoids biosynthesis.

The present study was designed to test the capacity of *H. procumbens* to alter eicosanoid metabolism so as to identify possible mechanisms which contribute to its antiinflammatory properties. Therefore we have investigated the production of PGE<sub>2</sub>, thromboxane (TX)B<sub>2</sub>, and 6-keto-PGF<sub>1α</sub> (as reflects of cyclooxygenase enzyme system) and LTB<sub>4</sub> (as reflect of 5-lipoxygenase enzyme system) by blood clotting spontaneously at 37 °C in vitro. Blood was obtained from healthy humans before and after 3 consecutive weeks of placebo or *H. procumbens* intake. This model is generally regarded as one of the best models to investigate in vivo drug effects on arachidonic acid cascade, because it takes into account the complex interactions between the different cell type capable modulating eicosanoid synthesis and metabolism (11).

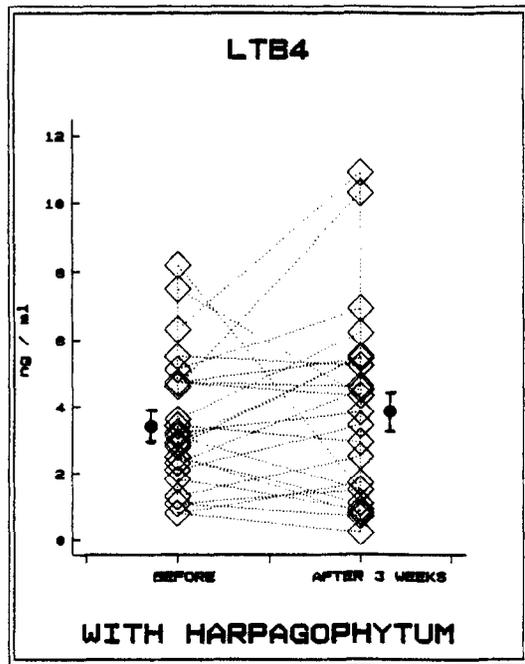
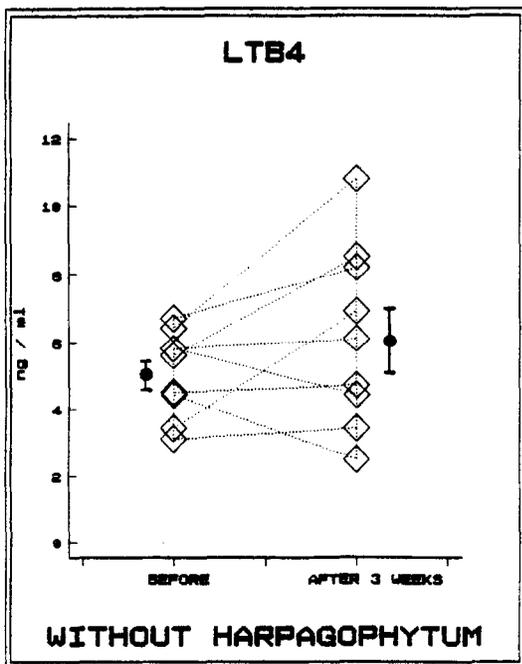
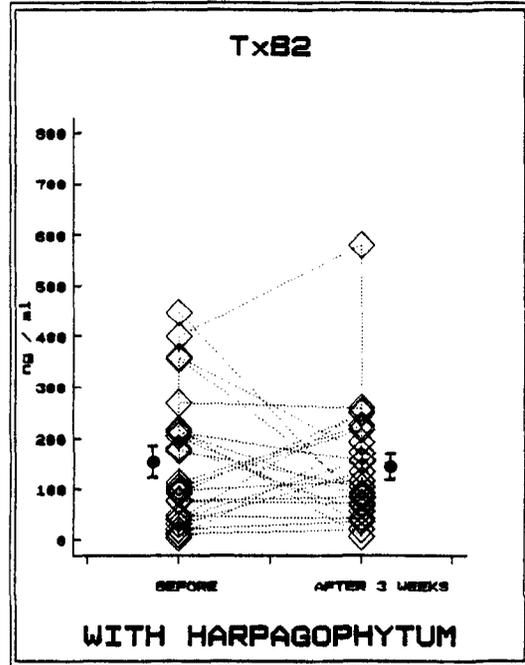
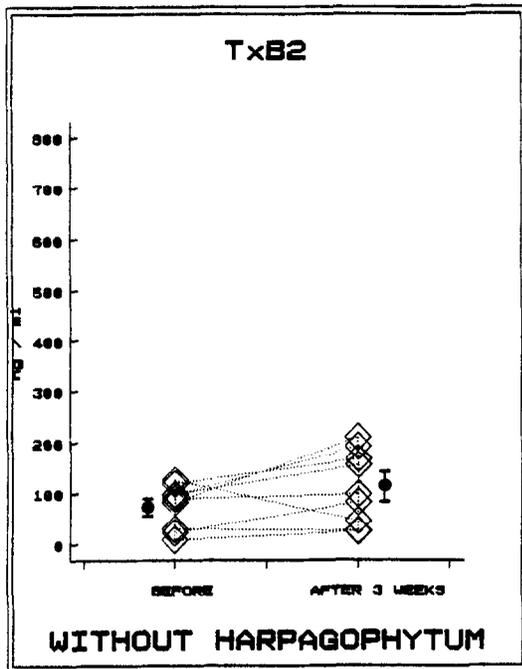
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**MATERIALS AND METHODS**

**Whole blood incubation**

Drug-free healthy human volunteers taking part in this study (age range: 20–35 years) were divided into two groups: control group (n = 9) and *H. procumbens* group (n = 25). Blood was obtained by venipuncture before and after 3 weeks intake of placebo for the first group

and *H. procumbens* (at the recommended dose: daily 4 capsules of 500 mg powder containing 3% of total glucosiridoids) for the second group. Blood (ca. 5 ml) was collected in glass tubes, allowed to clot by incubating at 37 °C for 1 h, and centrifuged at 3000 rpm for 10 min. Serum was stored at –20 °C until the time of the assay.



**Figure** Serum TXB<sub>2</sub> (as major cyclooxygenase product) and LTB<sub>4</sub> (as 5-lipoxygenase product) levels (ng/ml) after 1 h spontaneous clotting of human whole blood. Concentrations were measured before and after 3 weeks placebo (noted as *without Harpagophytum*) or *H. procumbens* intake (noted as *with Harpagophytum*).  
 ○ : individual value, ● : mean value + or – SEM; dotted lines join values from a same subject.

### Prostanoid extraction

3H-PGE<sub>2</sub> (5000 dpm) in 10 µl ethanol was added to 0.5 ml serum as internal standard for analytical recovery. The sample was acidified to pH 3.5 with 1M citric acid. The C18 bonded-phase packing cartridge (SepPak C18 Waters) was preconditioned with 15 ml ethanol followed by 15 ml water. After application of the sample, the cartridge was washed with 15 ml ethanol: water (15:85 V:V) and 20 ml petroleum ether. Prostanoids were eluted with 8 ml ethyl acetate, which was evaporated under nitrogen. The dry residue was stored at -25 °C until analysed.

### LTB<sub>4</sub> extraction

3H-LTB<sub>4</sub> (5000 dpm) was added to 0.5 ml serum as internal standard. The SepPak C18 cartridge was preconditioned with 10 ml water, followed by 10 ml methanol. After application of acidified sample, the column was washed with 10 ml water, 10 ml methanol: water (25:75 v:v), 10 ml water, and 10 ml hexane. LTB<sub>4</sub> was eluted with 7 ml ethyl acetate, which was evaporated under nitrogen. The dry residue was stored at -25 °C until analysed.

### Eicosanoid determination

PGE<sub>2</sub>, TXB<sub>2</sub>, 6-keto-PGF<sub>1α</sub> and LTB<sub>4</sub> were quantified by radioimmunoassay employing commercially available kits (from Pasteur Institute for PGE<sub>2</sub> and TXB<sub>2</sub>, and from Amersham for 6-keto-PGF<sub>1α</sub> and LTB<sub>4</sub>). Efficiency of elution of eicosanoids in our systems approaches 80%. Results were expressed in ng/ml serum. Values were reported as means (standard error of mean, SEM). Differences were analysed with Student's t-test for paired and unpaired data.

## RESULTS

Using the spontaneously clotting whole human blood model, one can simultaneously measure the *in vivo* effects of drugs on both cyclooxygenase and lipoxygenase pathways. Concentrations of TXB<sub>2</sub> were, as expected, the highest of the four eicosanoids measured in serum after 1 h blood clotting. The Figure shows TXB<sub>2</sub> and LTB<sub>4</sub> concentrations measured before and after 3 weeks placebo (noted as without Harpagophytum) or *H. procumbens* intake (noted as with Harpagophytum). The results (ng/ml, mean(sem)) were as follows: *without Harpagophytum group*, before placebo intake, PGE<sub>2</sub>: 1.9 (0.4) (n = 9), TXB<sub>2</sub>: 74 (14) (n = 9), 6-keto-PGF<sub>1α</sub>: 2.5 (0.9) (n = 9), LTB<sub>4</sub>: 5.0 (0.4) (n = 9), and after placebo intake, PGE<sub>2</sub>: 2.9 (0.9), TXB<sub>2</sub>: 111 (24), 6-keto-PGF<sub>1α</sub>: 2.1 (0.5), LTB<sub>4</sub>: 6.2 (0.9); *with Harpagophytum group*, before drug intake, PGE<sub>2</sub>: 2.1 (0.4) (n = 25), TXB<sub>2</sub>: 147 (27) (n = 25), 6-keto-PGF<sub>1α</sub>: 4.4 (0.7) (n = 13), LTB<sub>4</sub>:

3.4 (0.4) (n = 25), and after drug intake; PGE<sub>2</sub>: 3.2 (0.6), TXB<sub>2</sub>: 143 (24), 6-keto-PGF<sub>1α</sub>: 4.2 (0.9), LTB<sub>4</sub>: 3.8 (0.6). No statistically significant differences were seen, either between placebo and Harpagophytum groups before drug intake, or between before and after placebo or Harpagophytum intake, each subject serving as her own control.

## DISCUSSION

In spite of the widespread use of Devil's Claw in the treatment of a variety of diseases, including rheumatoid arthritis (1), the antiinflammatory and antiarthritic activity of this drug is far from proved. Some investigations of *H. procumbens* in animal models of inflammation and arthritis have been carried out (2–6). Considering their results, it is difficult to decide if *H. procumbens* shows or not an antiinflammatory and antiarthritic activity. Only one study has examined the *in vitro* effect of a Devil's Claw extract on PG biosynthesis: no inhibition of *in vitro* biosynthesis from arachidonic acid was seen (6).

The present study is the first that reports investigation of *in vivo* effect of *H. procumbens* on eicosanoid production in human.

Biosynthesis of eicosanoids by spontaneously clotting whole blood is a rather complex process which, in contrast to isolated cell populations, does however permit the important cell-cell interactions and therefore rather closely resembles *in vivo* conditions. Indeed there has been a growing number of reports on eicosanoid generation by transcellular metabolism, and on cross-cell signaling which can modulate arachidonic acid metabolism, viz between different blood cells and cells of the vascular wall (12, 13). TXB<sub>2</sub> (the major arachidonic product) formation by this model is known to be triggered by endogenously formed thrombin. In this study, the blood platelet count was omitted because of its relatively minor contribution to the variation in TXB<sub>2</sub> production (14). Clotting whole blood was therefore selected as a fluid phase model tissue where serum eicosanoids are not physiological parameters but rather reflect biosynthetic capacities and arachidonic acid as target in pharmacological mechanisms.

Our results indicate that, at least in healthy humans and in the selected conditions, Devil's Claw did not alter the eicosanoid biosynthesis, either by cyclooxygenase or by 5-lipoxygenase pathways.

In conclusion, the antiinflammatory and antiarthritic activity of *H. procumbens*, if any, take place independently of arachidonic acid metabolism alteration.

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