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## Selective COX-2 Inhibition by a *Pterocarpus marsupium* Extract Characterized by Pterostilbene, and its Activity in Healthy Human Volunteers

### Abstract

In this study, an extract of *Pterocarpus marsupium* Roxb. containing pterostilbene has been evaluated for its PGE<sub>2</sub>-inhibitory activity in LPS-stimulated PBMC. In addition, the COX-1/2 selective inhibitory activity of *P. marsupium* (PM) extract was investigated. Biological activity, as well as safety of PM extract was evaluated in healthy human volunteers. PM extract, pterostilbene and resveratrol inhibited PGE<sub>2</sub> production from LPS-stimulated human peripheral blood mononuclear cells (PBMC) with IC<sub>50</sub> values of 3.2 ± 1.3 µg/mL, 1.0 ± 0.6 µM and 3.2 ± 1.4 µM, respectively. When pterostilbene content of PM extract is calculated, PGE<sub>2</sub> production inhibition of PM extract is comparable to PGE<sub>2</sub> production inhibition of purified pterostilbene. Furthermore, in a COX-1 whole blood assay (WBA) PM extract was not effective while in a COX-2 WBA, PM extract decreased PGE<sub>2</sub> production indicating COX-2 specific inhibition. In healthy human volunteers, the oral use of 450 mg PM extract did not decrease PGE<sub>2</sub> production *ex vivo* in a WBA. Pterostilbene levels in serum were increased, but were 5-fold lower than the observed IC<sub>50</sub> for PGE<sub>2</sub> inhibition in LPS-stimulated PBMC. No changes from base-line

of the safety parameters were observed and no extract-related adverse events occurred during the study.

In conclusion, this is the first study to describe the selective COX-2 inhibitory activity of a *Pterocarpus marsupium* extract. Moreover, the PGE<sub>2</sub> inhibitory activity of PM extract was related to its pterostilbene content. In humans, 450 mg PM extract resulted in elevated pterostilbene levels in serum, which were below the active concentration observed *in vitro*. In addition, short-term supplementation of 450 mg PM extract is considered to be a safe dose based on the long history of use, the absence of abnormal blood cell counts and blood chemistry values and the absence of extract-related adverse events. This strongly argues for a dose-finding study of PM extract in humans to corroborate the *in vitro* observed inhibitory activity on PGE<sub>2</sub> production in order to resolve the potential use of PM extract in inflammatory disorders and/or inflammatory pain.

### Key words

*Pterocarpus marsupium* · Fabaceae · pterostilbene · resveratrol · PGE<sub>2</sub> · cyclooxygenase · healthy human volunteers

### Introduction

Cyclooxygenase (COX) plays an important role in inflammation and contributes to inflammatory pain. It is the rate-limiting enzyme in the conversion of arachidonic acid (AA) to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Different COX isoforms are known, COX-1 and COX-2 [1], [2] and more recently a third isoform, COX-3, was

identified, which appeared to be an alternative splicing variant of COX-1, present in the brain and heart [3]. By non-selectively inhibiting COX-1/2, non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain and suppress inflammation in a variety of diseases, such as osteoarthritis (OA) and rheumatoid arthritis (RA).

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Received July 19, 2004 · Accepted December 12, 2004

### Bibliography

Planta Med 2005; 71: 387–392 · © Georg Thieme Verlag KG Stuttgart · New York  
DOI 10.1055/s-2005-864130  
ISSN 0032-0943

COX-1 is expressed constitutively in many cells where it produces the prostanoids involved in homeostatic function, e.g., gastric cytoprotection and platelet activation. COX-2 is generally considered to be the inducible form and plays an important role during inflammation, because it accounts for most of the PGE<sub>2</sub> that is produced during inflammation. Chronic use of NSAIDs results in gastrointestinal toxicity [4] which is associated with their COX-1 inhibitory activity. This phenomenon has led to the development of selective COX-2 inhibitors [5], [6].

Resveratrol (Fig. 1) is a well-known member of the stilbenes and is found in grapes and other foods. Resveratrol has COX inhibitory properties or decreases the production of PGE<sub>2</sub> [7], [8], [9], [10]. Another member of the stilbenes is pterostilbene (Fig. 1), which is a major constituent of a commercially available *Pterocarpus marsupium* (PM) extract. *P. marsupium* Roxb. (Fabaceae) is a plant that has been used in Indian traditional medicine. A limited number of papers review its anti-diabetic potential [11], [12], [13]. However, herbal extracts typically contain a variety of constituents that may have different biological effects or could contribute to the same biological effect that is observed. In the present study, it was evaluated whether PM extract is a potential candidate for intervention strategies targeting chronic inflammatory disorders and/or inflammatory pain. PGE<sub>2</sub> inhibitory activity of PM extract is demonstrated using lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells (PBMC) and this was related to the pterostilbene content of PM extract. Furthermore, COX-1/2 selective inhibition by the PM extract in a COX-1 whole blood assay (WBA) and a COX-2 WBA was investigated. Finally, in healthy human volunteers, the *in vivo* PGE<sub>2</sub> inhibitory activity of PM extract, several safety parameters and the bioavailability of pterostilbene were evaluated.

## Materials and Methods

All incubations of cells were done at 37 °C in a humidified environment containing 5% CO<sub>2</sub>.

### Characterization of PM extract by HPLC

Fig. 2 shows the HPLC chromatograms of PM extract, pterostilbene and resveratrol that were used in this study. In short, samples were dissolved in dimethyl sulfoxide (DMSO) (100 mg/mL PM extract, 3 mg/mL pterostilbene and 1 mg/mL resveratrol) and 10 µL was injected onto a Superspher 100 reverse phase C18 HPLC column (125 × 3 mm, 4.0 µm, Bischoff, Leonberg, Germany) and eluted with 0.01% trifluoroacetic acid with increasing concentrations of acetonitrile [at 4.7–44.6 minutes from 0 to 25% acetonitrile (linear), at 44.6–54.9 minutes from 25% to 51.3% acetonitrile (linear) and at 54.9–64.8 minutes from 51.3% to 100% acetonitrile until 76.5 minutes]. UV absorbance was recorded at 220 nm, 254 nm and 280 nm.

### LPS-stimulated PBMC and metabolic activity assay

PBMC from buffy coats (Sanquin blood bank, Amsterdam, The Netherlands) of human blood from healthy donors were isolated and stored in liquid nitrogen and subsequently thawed using standard procedures. 150 µL/well PBMC in cell culture medium (RPMI-1640 containing 25 mM HEPES and 2 mM L-glutamine (Life-Technologies, Merelbeke, Belgium) and further enriched

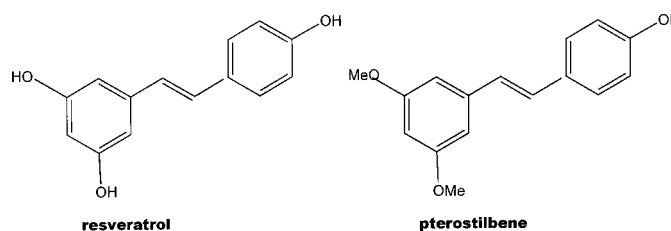
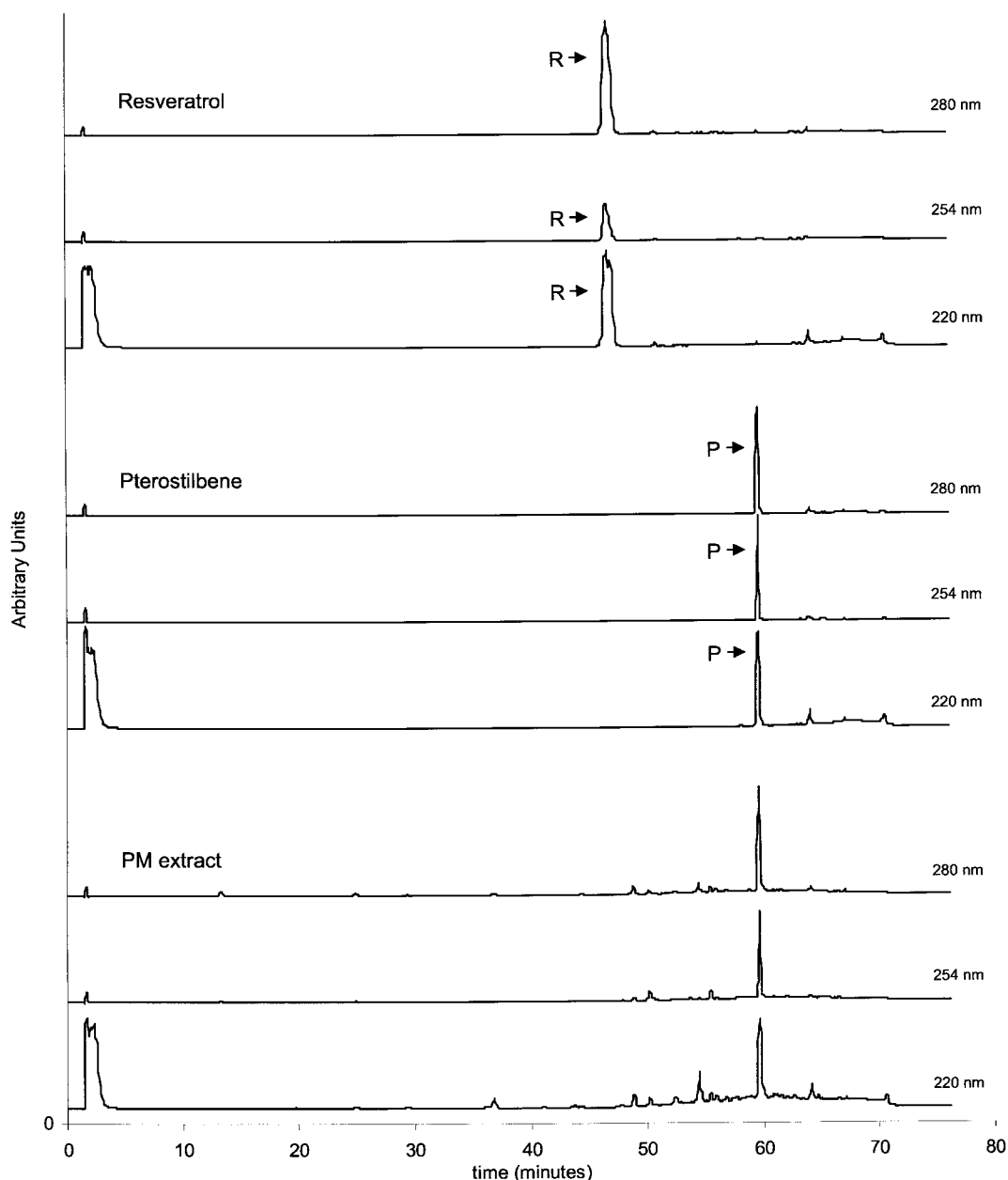


Fig. 1 Chemical structures of resveratrol and pterostilbene.

with 100 U/mL penicillin/streptomycin, 1.0 mM sodium pyruvate and 10% heat-inactivated fetal calf serum) were pipetted into a 96-well flat bottom microtiter plate (BD Falcon, Erembodegem Aalst, Belgium) in a concentration of  $1.5 \times 10^5$  cells. PM extract (Silbinol™, commercially available extract from *P. marsupium* heartwood and bark containing 5.5% pterostilbene (w/w) as determined by HPLC, Sabinsa, Piscataway, NJ, USA), pterostilbene (purified from *P. marsupium*, > 95%, Chromadex, Santa Anna, CA, USA) and resveratrol (> 99%, Sigma, Zwijndrecht, The Netherlands) were dissolved in DMSO and subsequently further diluted in cell culture medium and added to the cells in an individual concentration range. DMSO concentration in the wells was 0.1%. In control experiments this concentration did not show any effects on the measured parameters. Subsequently the cells were incubated for one hour (volume per well 170 µL). After one hour, LPS (*E. coli* B55:O55, Sigma) was added resulting in a final concentration of 10 ng/mL LPS and a final volume of 200 µL. The cells were incubated for 20 hours. At the end of the 20 hour incubation period the supernatants were harvested and stored at –80 °C until further analysis. Next to the plates for PGE<sub>2</sub> analysis, identical plates were used to determine metabolic activity of the PBMC with and without the presence of test agent using the WST-1 assay (Roche Diagnostics, Almere, The Netherlands). WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenedisulfonate} is a substrate for the enzyme succinate dehydrogenase. The conversion product formazan can be measured at 450 nm with a reference filter at 655 nm. The activity of succinate dehydrogenase reflects mitochondrial activity and may therefore be indicative for metabolic activity and/or cell viability. After the 1 hour pre-incubation period of PBMC and test agents or vehicle and the subsequent 20 hour incubation with LPS, WST-1 was added undiluted (10 µL/well). Absorbance was measured using a plate reader (Ultramark, BioRad, Veenendaal, The Netherlands) directly after WST-1 addition and after 5 hours incubation of WST-1 with the cells. Control values (without test agents) were set at 100% and all values were expressed as percentage of control values.

### COX-1 and COX-2 whole blood assay (WBA)

After obtaining written informed consent, ten mL blood from healthy human volunteers were collected by venupuncture into heparin (17 IU/mL). COX-1 and COX-2 WBA were performed according to Warner et al. [14]. In short, for the COX-1 assay, test agents or vehicle were added and incubated with whole blood after which the calcium ionophore A23187 was added for 30 minutes. For the COX-2 assay, aspirin was added to inactivate COX-1 after which the test agents or vehicle were added plus LPS for another 18 h. SC-560 (Calbiochem, VWR International, Amsterdam, The Netherlands) and celecoxib (content of Celebrex



**Fig. 2** HPLC chromatogram of PM extract (lower three lines), pterostilbene (middle three lines) and resveratrol (upper three lines) recorded at 220, 254 and 280 nm. With the arrows resveratrol (R) and pterostilbene (P) peaks are indicated.

capsule containing 200 mg celecoxib was corrected for its excipient content based on weight; Pfizer, local pharmacy) were used as positive controls for a selective COX-1 and a selective COX-2 inhibitor respectively. Concentration DMSO (vehicle) was 0.1% in all wells. Plates were centrifuged and supernatants were harvested and stored at  $-80^{\circ}\text{C}$  until analysis.

#### Activity of orally administered PM extract in healthy human volunteers

To evaluate the activity of PM extract in humans when administered orally, a healthy human volunteer study was performed according to an open, randomized, positive-controlled design with three parallel groups. Whole blood was stimulated *ex vivo* with LPS and production of  $\text{PGE}_2$  was analyzed without knowing the donor identification and group distribution. The same procedure was followed when analyzing  $\text{TXB}_2$  in serum. Thirteen healthy human volunteers (see Table 1 for subject demographics) were randomized to receive one of the following treatments: A)

450 mg PM extract once daily ( $n = 5$ ); B) 225 mg PM extract twice daily, in the morning and in the evening ( $n = 5$ ); C) 200 mg celecoxib (Celebrex capsule containing 200 mg celecoxib) once daily as a positive control for selective COX-2 inhibition ( $n = 3$ ). The dose of 450 mg oral intake daily was based upon a pilot mouse study in which 1.25 mg PM extract once daily for ten days resulted in decreased  $\text{PGE}_2$  production of *ex vivo* stimulated whole blood (data not shown). Conversion of animal dosage to human dosage is done taking into account the ratio of metabolic weight, which is defined as  $(\text{body weight in kg})^{0.75}$  [15]. Together with the supplier's information 450 mg was considered the appropriate daily dose for a first-time pilot in healthy human volunteers.

The study was approved by the ethics committee (BEBO) and conducted according to the principles of the 'Declaration of Helsinki' (52nd WMA General Assembly, Edinburgh, Scotland, October 2000) and according to the Dutch law on scientific medical

Table 1 Subject demographics and baseline characteristics. Values are mean  $\pm$  SD

	A: 450 mg PM extract once daily (n = 5)	B: 225 mg PM extract twice daily (n = 5)	C: 200 mg Celecoxib once daily (n = 3)
Male:female	3:2	2:3	1:2
Age (years)	27 $\pm$ 8	26 $\pm$ 8	33 $\pm$ 15
BMI (kg/m <sup>2</sup> )	22.5 $\pm$ 2.0	23.5 $\pm$ 3.3	23.6 $\pm$ 4.7

research with humans in the Netherlands. Prior to study enrolment, written informed consent was obtained from each subject.

Blood was taken before supplementation at day 0 and at days 2, 7 and 14 after the start of supplementation and WBAs were performed at these days. Blood was drawn into heparin (17 IU/mL) by venupuncture 3 hours after intake of the daily dose for groups A and C or half the daily dose for group B. Heparinized blood was aliquoted in 100  $\mu$ L volumes in a 96-well plate. 100  $\mu$ L cell culture medium (without serum) was added containing LPS (100 ng/mL) and plates were incubated for 20 hours. Plates were centrifuged and supernatants were harvested and stored at  $-80^{\circ}\text{C}$  until analysis. Blood samples for TXB<sub>2</sub> analysis in serum were drawn by venupuncture into 5 mL glass vacutainers and allowed to clot for one hour at  $37^{\circ}\text{C}$  in a water bath after which the sera were stored at  $-80^{\circ}\text{C}$ . For the evaluation of safety parameters, blood was drawn at day 0 and at day 14 of supplementation. Erythrocyte sedimentation rate, hemoglobin, hematocrit, white blood cell count, urea, creatinine, Na, K, Cl, gamma-glutamyl-transpeptidase (gamma-GT), alkaline phosphatase, bilirubin and liver aminotransferases AST and ALT were determined at baseline and 14 days after supplementation.

### Measurement of pterostilbene in serum

Serum samples were centrifuged at 13 000 rpm using an eppendorf centrifuge. Supernatant (500  $\mu$ L serum) was incubated in 10 mL glass centrifugation tubes with 100  $\mu$ L  $\beta$ -glucuronidase from *Helix pomatia* (2000 U/mL, Sigma, Zwijndrecht, The Netherlands) and 400  $\mu$ L sodium acetate solution (1 M, pH 5.0) for 24 hours at  $37^{\circ}\text{C}$ . The aglycone was extracted for 5 minutes with 2 mL *tert*-butyl methyl ether containing 1  $\mu$ g 4-hydroxybenzophenone (98%, Sigma, Zwijndrecht, The Netherlands) as an internal standard. Tubes were centrifuged for 5 minutes at 3000 rpm (Sorvall RT7) and the upper layer was transferred to another tube. Vacuum dried residue was dissolved in 150  $\mu$ L ethanol and transferred to a 300  $\mu$ L microvial (Alltech, Breda, The Netherlands). To precipitate any fat the vials were placed at  $4^{\circ}\text{C}$  overnight. Subsequently, the vials were centrifuged for 5 minutes at 3000 rpm and  $4^{\circ}\text{C}$  (Sorvall RT7). 10  $\mu$ L samples were injected onto a Superspher 100 reverse phase C18 HPLC column (125  $\times$  3 mm, 4.0  $\mu$ m, Bischoff, Leonberg, Germany) and eluted for 16 minutes with 40% (v/v) acetonitrile in water with retention times for the internal standard of 4.2 minutes and for pterostilbene 13.7 minutes. The peaks of the internal standard and pterostilbene were measured by UV absorption at 306 nm after which the concentration of pterostilbene was calculated.

### Eicosanoids measurement

PGE<sub>2</sub> and TXB<sub>2</sub> were measured in the thawed supernatants and sera, respectively, using a commercial enzyme immunoassay (Biotrak Amersham, Buckinghamshire, UK) according to the manufacturer's protocol (protocol 2 for PGE<sub>2</sub>).

### Statistical analyses

IC<sub>50</sub> values from LPS-stimulated PBMC were determined using a sigmoidal dose response curve using Prism4<sup>®</sup> software from Graphpad. PGE<sub>2</sub> data from COX-1 and COX-2 WBA were analyzed by linear regression after log transformation of the concentrations of the different agents used [ $\ln(\text{concentration} + 1)$ ]. The average of the slopes of 5 human donors was statistically tested to be  $< 0$  with a one sample T-test in SPSS<sup>®</sup>. Pterostilbene concentrations in plasma of healthy human volunteers were analyzed by ANOVA and post-hoc a Tukey-Kramer multiple comparison test was performed.

### Results

PM extract, pterostilbene and resveratrol (for pterostilbene and resveratrol see Fig. 1) all dose-dependently inhibited PGE<sub>2</sub> production of 20 hour LPS-stimulated PBMC. IC<sub>50</sub> values are  $3.2 \pm 1.3$   $\mu$ g/mL,  $1.0 \pm 0.6$   $\mu$ M and  $3.2 \pm 1.4$   $\mu$ M for PM extract, pterostilbene and resveratrol, respectively (shown in Table 2). For PM extract, there is no decrease in metabolic activity at the IC<sub>50</sub> value. At the IC<sub>50</sub> value for pterostilbene there is a small decrease of metabolic activity of 14% and for resveratrol the decrease is 10%.

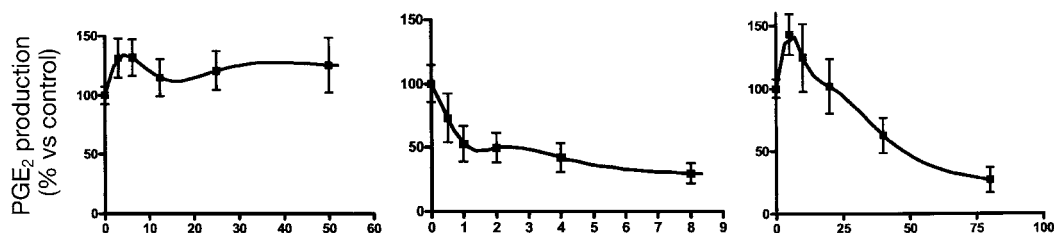
To differentiate whether PGE<sub>2</sub> inhibition of PM extract results from COX-1 or from COX-2 inhibition a WBA is used. PM extract was not able to inhibit PGE<sub>2</sub> after COX-1 stimulation. Both celecoxib and SC-560 dose-dependently inhibited the PGE<sub>2</sub> production from the COX-1 assay, which was statistically significant. In the COX-2 assay, SC-560 did not inhibit the PGE<sub>2</sub> production. PM extract and celecoxib dose-dependently inhibited the PGE<sub>2</sub> production, both statistically significant (Fig. 3), indicating COX-2 specific inhibition.

In order to get insight into the *in vivo* effects of orally administered PM extract a healthy human volunteer study was performed. PGE<sub>2</sub> was inhibited by celecoxib from day 2 to day 14 by 75% or more. No inhibition of PGE<sub>2</sub> was observed in the PM extract administered subjects (data not shown). TXB<sub>2</sub> values of serum in the three supplementation groups did not change from baseline (data not shown). Serum concentrations of pterostilbene at day 0 were below the detection limit of 5  $\mu$ g/L plasma (20 nM). At days 2, 7 and 14 the pterostilbene levels were in-

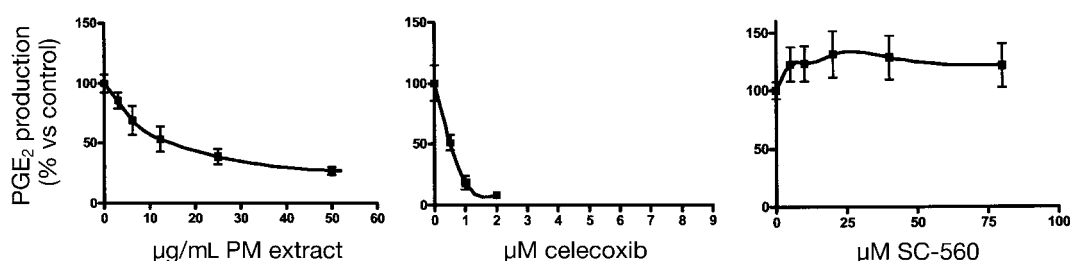
Table 2  $IC_{50}$  values for  $PGE_2 \pm SD$  from 20 h LPS-stimulated PBMC and the decrease in metabolic activity at the determined  $IC_{50}$  values

	$IC_{50} PGE_2$	% Decrease in WST at $IC_{50}$
PM extract	$3.2 \pm 1.3 \mu\text{g/mL}$ (n = 6)	No decrease
Pterostilbene	$1.0 \pm 0.6 \mu\text{M}$ (n = 3)	$14\% \pm 0.13$
Resveratrol	$3.2 \pm 1.4 \mu\text{M}$ (n = 3)	$10\% \pm 0.09$

### A. COX-1 Assay



### B. COX-2 Assay



$PGE_2$  values in  $\mu\text{g/mL}$  and subsequently one sample T-tests are done to test whether the slopes are  $< 0$ . In the COX-1 assay PM extract did not demonstrate statistically significant  $PGE_2$  inhibition as opposed to SC-560 ( $P < 0.05$ ) and celecoxib ( $P < 0.05$ ). However, in the COX-2 assay, PM extract showed statistically significant  $PGE_2$  inhibition ( $P < 0.05$ ). Expectedly, celecoxib inhibited  $PGE_2$  ( $P < 0.05$ ). Also SC-560, which is a selective COX-1 inhibitor, did not inhibit  $PGE_2$  from COX-2 ( $P > 0.05$ ). These results indicate specific COX-2 inhibition by PM extract.

creased significantly versus day 0, although no significant difference exists between the different days (Table 3).

From a questionnaire regarding some major body systems the relationship of the adverse events and the PM extract was documented. There was no occurrence of PM extract-related adverse events. In addition, there were no changes from baseline in hematology and blood chemistry values (erythrocyte sedimentation rate, hemoglobin, hematocrit, white blood cell count, urea, creatinine, Na, K, Cl, gamma-glutamyl-transpeptidase (gamma-GT), alkaline phosphatase, bilirubin and liver aminotransferases AST and ALT) after 14 days of PM extract supplementation in either the group receiving 450 mg PM extract once daily or the group receiving 225 mg PM extract twice daily.

### Discussion

This is the first study to describe the COX-2 selective inhibitory activity of an extract from *Pterocarpus marsupium* Roxb. (PM extract). In addition, this present study describes that the  $PGE_2$  inhibitory activity of PM extract is related to its pterostilbene content. *P. marsupium* is a plant used in Indian traditional medicine to which anti-diabetic properties have been ascribed (reviewed in [11], [12], [13]). In this present study, a  $PGE_2$  inhibitory effect

Fig. 3  $PGE_2$  production is expressed as percentage of control values ( $PGE_2$  value at no agent incubated) and cubic spline curves are shown to visualize data. **A**  $PGE_2$  production from 30 minutes A23187-stimulated human whole blood with PM extract and celecoxib and SC-560 as controls. **B**  $PGE_2$  production from 6 hours aspirin pretreated and subsequent 18 hours LPS-stimulated human whole blood and incubated during the last 18 hours with PM extract and celecoxib and SC-560 as controls. Data represent average of 5 human donors  $\pm$  SEM. Linear regression is performed upon absolute

of a commercially available extract of *P. marsupium*, characterized by pterostilbene, was demonstrated. Pterostilbene is a structure analogue of resveratrol (Fig. 1) that has been reported to inhibit COX-1 and/or COX-2 [8], [10]. The COX isoform enzyme activity of resveratrol and its structure analogue pterostilbene was studied earlier by Rimando et al. [16]. In that study an  $IC_{50}$  value of resveratrol for both COX-1 and COX-2 of about  $1 \mu\text{M}$  was reported. In addition, the COX inhibitory activity of pterostilbene was reported to be much lower in that same study: the  $IC_{50}$  value for COX-1 was  $19.8 \mu\text{M}$  and for COX-2 even  $83.9 \mu\text{M}$  [16]. However, in the present study, pterostilbene showed a much higher activity. The activity was even higher compared to the activity of resveratrol (Table 2). In the present study human LPS-stimulated PBMC were used to evaluate inhibition of  $PGE_2$  production in contrast with the study described earlier, in which COX enzyme activity assays were used with sheep COX-1 and human recombinant COX-2 (Rimando, personal communication). The different experimental set-ups might be an explanation for the difference in the observed activities.

If pterostilbene would be the main contributing factor for the decreased  $PGE_2$  production by PM extract at an  $IC_{50}$  value of  $3.2 \pm 1.3 \mu\text{g/mL}$ , the calculated  $IC_{50}$  value of pterostilbene would be  $0.69 \pm 0.29 \mu\text{M}$ . The measured  $IC_{50}$  value of pterostilbene is  $1.0 \pm 0.6 \mu\text{M}$  (Table 2). The measured and calculated  $IC_{50}$  values of 0.69

Table 3 Pterostilbene concentrations in plasma of healthy human volunteers

	Concentration pterostilbene in plasma (nM) $\pm$ SEM (n = 5)			
	Day 0	Day 2	Day 7	Day 14
A: PM extract 450 mg once daily	< 20	176 $\pm$ 50	196 $\pm$ 21	144 $\pm$ 20
B: PM extract 225 mg twice daily	< 20	85 $\pm$ 9	98 $\pm$ 7	101 $\pm$ 20

Statistical analysis was done by ANOVA and post-hoc a Tukey-Kramer multiple comparison test was done. At days 2, 7 and 14 the pterostilbene levels are significantly increased versus day 0 (minimum detection limit was used at day 0). Between days 2, 7 and 14 there is no statistically significant difference.

and 1.0 are not significantly different (unpaired t-test). This suggests that pterostilbene contributes for a large part to the PGE<sub>2</sub> inhibitory activity of PM extract as seen in LPS-stimulated PBMC. From the results of the COX-1 (A23187-stimulated) and COX-2 (aspirin pre-treated and subsequently LPS-stimulated) WBAs it can be concluded that PM extract inhibits PGE<sub>2</sub> production that is derived from COX-2 and not PGE<sub>2</sub> production that is derived from COX-1 (Fig. 3) indicating a COX-2 selective inhibition. In humans, PM extract at the given dose of either 450 mg PM extract daily or 225 mg PM extract twice daily was not orally active. Pterostilbene levels in serum, however, were increased when compared to baseline levels. The highest concentration observed of pterostilbene in serum (196 nM) is 5-fold lower than the IC<sub>50</sub> of pterostilbene for PGE<sub>2</sub> inhibition when added to LPS-stimulated PBMC (1.0  $\mu$ M). This might explain why in the healthy volunteers there was no effect of PM extract on PGE<sub>2</sub> production.

In conclusion, the present study describes the PGE<sub>2</sub> inhibitory activity of PM extract that appears to be closely associated with its pterostilbene content. Moreover, PM extract exhibits COX-2 selective inhibitory activity *in vitro*. These data suggest that PM extract and, in particular, pterostilbene are interesting candidates for intervention strategies in inflammatory diseases and/or inflammatory pain. However, 450 mg PM extract daily administered to humans, results in pterostilbene levels in serum too low to achieve PGE<sub>2</sub> inhibitory activity. Together with the observation that PM extract at 450 mg daily is considered a safe dose, based on the long history of use, the absence of abnormal blood cell counts and blood chemistry values, and the absence of extract-related adverse events, this strongly argues for a dose-finding study with a higher daily dose in humans to corroborate the inhibitory activity on PGE<sub>2</sub> production.

### Acknowledgements

The authors wish to thank the phlebotomists C. H. Rouws, M. E. H. de Lange and M. P. Verheijen for assisting and M.T.R. Jansen for coordinating the healthy human volunteer study, G. de Vrij and E. Voogd for HPLC analyses and Dr. L. R. Verdooren for assisting with statistical data analysis.

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