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Anti-inflammatory activity of Grains of paradise (*Aframomum melegueta* Schum) extract

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ABSTRACT: The ethanolic extract of grains of paradise (*Aframomum melegueta* Schum, *Zingiberaceae*) has been evaluated for inhibitory activity on cyclooxygenase-2 (COX-2) enzyme, *in vivo* for the anti-inflammatory activity and expression of several pro-inflammatory genes. Bioactivity guided fractionation showed that the most active COX-2 inhibitory compound in the extract was [6]-paradol. [6]-Shogaol, another compound from the extract, was the most active inhibitory compound in pro-inflammatory genes expression assays. In a rat paw edema model, the whole extract reduced inflammation by 49% at 1000 mg/kg. Major gingerols from the extract [6]-paradol, [6]-gingerol, and [6]-shogaol reduced inflammation by 20%, 25% and 38% respectively when administered individually at a dose of 150 mg/kg. [6]-shogaol efficacy was at the level of aspirin, used as a positive control. Grains of paradise extract has demonstrated an anti-inflammatory activity, which is in part due to the inhibition of COX-2 enzyme activity and expression of pro-inflammatory genes.

KEYWORDS: anti-inflammatory*,* Grains of paradise*, Aframomum melegueta* Schum, *Zingiberaceae*, gingerols, COX-2, paw edema

INTRODUCTION

Inflammation is a localized response of a tissue to injury, caused by a mechanical or biological agent or by an aberrant autoimmune response[1]. The arachidonic acid pathway constitutes one of the main cellular mechanisms for mediating inflammation. This pathway includes the cyclooxygenase pathway and the 5-lipoxygenase pathway. Prostaglandins are the end products of the cyclooxygenase pathway. The enzymes involved in prostaglandin synthesis and the receptors to which prostaglandins bind are well-known pharmacological targets. For example, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) reduce inflammation by reducing prostaglandin synthesis by inhibiting cyclooxygenase. In humans, cyclooxygenase is present in at least two isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is expressed constitutively in most tissues and performs many 'housekeeping' functions, such as maintaining the protective lining of the stomach, regulating blood flow through the kidneys, and promoting platelet aggregation, whereas COX-2 is an inducible isoform that is mainly produced in inflamed tissues[2].

Compounds that inhibit COX-2 activity or lower its expression are significant not only for the treatment of inflammatory responses, but also for human health and wellness in general[3,4]. In our search for botanical cyclooxygenase inhibitors with anti-inflammatory activity, we tested an ethanolic extract of grains of paradise (*Aframomum melegueta*) in *in vitro* and *in vivo* assays. This plant is a member of the ginger family (Zingiberaceae) and grows wild or is cultivated in tropical areas of Africa, notably West Africa. The plant seeds are used to flavor foods and as components of traditional African medicine. In medieval Europe they were a highly prized spice

that was eventually replaced by black pepper and other spices after the opening of the Asian trade routes. Ethnobotanically, the seed extract is used as a remedy against stomachache, diarrhea, and snakebite[5, 6]. Additionally there are reported studies on anti-ulcer, cytoprotective, and anti-microbial activities as well as a sexual performance enhancing effects of grains of paradise[7, 8, 9, 10, 11]. The aqueous seed extract has been shown to reduce the frequency of abdominal constrictions induced by acetic acid in mice and has significant anti-inflammatory activity[12]. It was later reported that the same extract has peripheral analgesic activity[12]. Additionally, it was suggested that the extract has membrane stabilizing activity along with anti-oxidant effects[13], as well as hypotensive and antihypertensive activity in humans[14]. Recently, it has been also found that extract has an effect on the whole-body energy expenditure and visceral fat in humans[15, 16].

The objectives of this study were to evaluate the anti-inflammatory effects of grains of paradise for inhibition of COX-2 enzyme activity, inhibition of expression of pro-inflammatory genes (*in vitro*) and anti-inflammatory activity (*in vivo)* in a rat paw edema assay, and to characterize active compound(s) from the extract that could be potentially beneficial in the treatment of inflammatory conditions.

MATERIALS AND METHODS

Chemicals and reagents. All chemicals and reagents were purchased from Sigma Aldrich Co. (St Louis, MO, USA) unless otherwise indicated. [6]-gingerol and [6]-shogaol (95% purity) were purchased from Dalton Chemical Laboratories Inc., (Toronto, ON, Canada). Aspirin (purity

>99%) and Vioxx ® (Merck & Co, Inc., West Point, PA, USA; purity >98%) were used as positive controls.

Plant material. Seeds of *Aframomum melegueta* were commercially purchased from Abidjan, Ivory Coast, and identified by Dr. L. Struwe, Rutgers University. A voucher specimen (Struwe 1424 CHRB) is deposited at the Chrysler Herbarium, Rutgers University, New Brusnwick, USA.

Preparation of the extract. Dry seeds were ground into powder and the seed powder (2 g) was extracted in 95% ethanol (20 mL) for 24 h at room temperature with continuous agitation provided by a platform shaker. After extraction, the sample was filtered and the solvent removed by rotary evaporation yielding 40.25 mg (2 %). The extract was dissolved in 95% ethanol to appropriate concentrations for *in vitro* assays, chemical characterization, and bioactivity guided fractionation.

COX-2 *in vitro* assay. Grains of paradise extract was dissolved in 95% ethanol at a concentration of 1 mg/mL. The activity of the extract was tested with a colorimetric COX (ovine) inhibitor screening assay (catalogue no. 760111, Cayman Chemical, Ann Arbor, MI, USA). The screening assay measures the peroxidase activity of cyclooxygenase by monitoring the appearance of oxidized N,N,N'N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm as previously described[12]. A relatively higher TMPD concentration corresponds to a greater absorbance at 590 nm, a greater peroxidase activity, and a greater COX-2 activity (i.e., less inhibition of COX-2 activity). The assays were performed according to manufacturer's instructions.

Bioassay guided purification of the extract. Purification and isolation of compounds were carried out using a preparatory HPLC from Waters Corp. (Milford, MA, USA) consisting of W717 plus auto sampler, W600E multi solvent delivery system, W600 controller, W490E multi

90 wavelength detector and Waters fraction collector. A Waters liquid chromatography – mass
91 spectrometry (LC-MS) Integrity™ system consisting of a solvent delivery system with a W616
92 pump and W600S controller, W717 plus auto-sampler, W996 PDA detector and Waters TMD
93 Thermabeam™ electron impact (EI) single quadrupole mass detector with fixed ionization
94 energy of 70 eV was used for analysis. Data were collected and analyzed with the Waters
95 Millennium® v. 3.2 software, linked with the 6th edition of the Wiley Registry of Mass Spectral
96 Data, containing 229,119 EI spectra of 200,500 compounds.
97
98 **Gene expression - Macrophage cell culture.** The mouse monocyte/macrophage cell line RAW
99 264.7 (ATCC TIB-71 obtained from American Type Culture Collection, Manassas, VA,
100 USA) was maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Inc., Carlsbad,
101 CA, USA) supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% heat-
102 inactivated fetal bovine serum (Sigma Aldrich Co). The cells were kept in a 37 ºC incubator
103 with 5% CO_2. Cells were sub-cultured by scraping when plates reached 90% confluence with a
104 1:5 ratio in fresh medium.
105 Cells were seeded at a density of $4x10^5$ cells per well (viable cell counts were carried out by
106 trypan blue staining using a hemocytometer) in 24-well plates 12 h prior to treatment. The cells
107 were then treated with test compounds dissolved in dimethyl sulfoxide (DMSO) at pre-
108 determined doses (based on MTS assays) for 2 h before elicitation with bacterial endotoxin
109 Llipopolysaccharide from 'E. coli', serotype 055:B5 (LPS) (Sigma Aldrich Co) at 1 µg/mL for
110 an additional 6 h. For every experiment one positive control (cells treated only with LPS and
111 vehicle) and one negative control (cells treated with vehicle only) were included. Three
112 replicates were made for both the treatments and the controls. Final concentration of DMSO in

cells was 1%. The same concentration was used as vehicle control. At the end of the treatment period, cells were harvested in Trizol reagent for subsequent cellular RNA extraction.

Gene expression - Cell viability assay and dose range determination. A Cell Titer 96 MTS assay kit (Promega Corp., Madison, WI, USA) was used to determine the relative number of viable cells remaining after incubation with treatments. The assay has been performed according to the manufacture's protocol and previously described method[18].. In short, the assay was performed by treating cells with different ectract concentrations, followed by adding 20 µL reagent 3-4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium/phenazine methosulfate (MTS/PMS) directly to culture wells, incubating for 3 h at 37°C., Plates were read using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 490 nm. For dose range studies, the highest non-toxic concentrations for respective compound/extract were selected.

Gene expression - Quantitative polymerase chain reaction (qPCR) and data analysis. The synthesized cDNAs were diluted 4-fold. Two microliters of each diluted sample were added to 0.5µL gene specific primers (6µM, oligos synthesized by IDT Inc., Coralville, IA, USA), 12.5µl of Brilliant SYBR green PCR master mix (2X) (Stratagene, La Jolla, CA, USA) containing green jump-start Taq ready mix and final volume was brought to 25 µL by adding sterile distilled water. Carboxy-X-rhodamine (ROX) was used as an internal dye. To avoid interference due to genomic DNA contamination, only intron-overlapping primers were selected using the Primer Express® vers. 2.0 software (Applied Biosystem, Foster City, CA, USA) as shown in Table 2. PCR amplifications were performed on MX3000p system (Stratagene, La Jolla, CA, USA) as described in [19]. RNA expressions for genes of interest, normalized with respect to the expression of housekeeping β-actin gene, were analyzed using the ΔΔCt method[19].

Carrageenan induced rat paw edema. Groups of Long Evans-derived male rats with body weights 150±10 g were housed 6 animals per cage in a room kept at 22-23 °C with 60-70% humidity and a 12 h light/dark cycle for 1 week prior to the start of the study. Access to standard lab chow and water was *ad libitum*. All aspects of this work including housing, experimentation and disposal of animals were performed in general accordance with the International Guiding Principles for Biomedical Research Involving Animals (CIOMS Publication No. ISBN 9290360194, 1985) and the protocol was approved by MDS Pharma (King of Prussia, PA, USA). The animals were fasted overnight prior to use.

Grains of paradise extract and pure compounds ([6]-paradol, [6]-shogaol, [6]-gingerol) were administered p.o. (extract at 1000 and 500 mg/kg and pure compounds at 150 mg/kg) suspended in 0.2% Tween-80/10% ethanol one hour before right hind paw injection of carrageenan (0.1 mL of 1% suspension intraplantar, TCI, Tokyo, Japan). Aspirin (150 mg/kg, Sigma Aldrich Co.) was used as a reference compound (positive control). The severity of the hind paw edema as a measure of inflammation was recorded 3 h after carrageenan administration using a plethysmometer (Ugo Basile, Comerio VA, Italy) with water cell (25 mm diameter). Reduction of edema was calculated by comparison with vehicle control. The anti-inflammatory activity is expressed as a percentage of edema reduction.

Statistical analysis. Statistical comparisons were performed between the vehicle control and treated groups. For the *in vitro* and *in vivo* assays, the results are presented as the mean ± SD (standard deviation) The statistical analysis was calculated by using analysis of variance (ANOVA) test and followed by post-hoc analysis using Tukey's Multiple Comparison Test.

RESULTS AND DISCUSSION

159

160 In a search for novel anti-inflammatory compounds we evaluated ethanolic extract of grains of
161 paradise (*Aframomum melegueta*) for inhibition of COX-2 enzyme activity, inhibition of pro-
162 inflammatory genes expression (*in vitro*), anti-inflammatory activity in rat paw edema assay (*in*
163 *vivo*) and the active compounds from the extract were characterized.

164

165 COX-2 enzyme inhibitory activity and active compounds characterization - at an initial
166 concentration of 1 mg/mL the ethanolic extract demonstrated inhibitory activity against COX-2,
167 similar to the drug Vioxx® that was used as a positive control. The level of inhibition for the
168 extract was 76% compared to 87% for Vioxx® (Table 1). Biochemical analytical data indicated
169 that the extract is a complex mixture of compounds. In order to identify compounds responsible
170 for the observed activity, the extract was subjected to bioactivity guided fractionation with
171 concurrent LC-MS analysis. Bioactivity guided fractionation was performed in the following
172 manner:
173 Grains of paradise extract (1 g, obtained from a large scale extraction) was dissolved in 95%
174 ethanol (20 mL) and fractionated using a preparatory HPLC. For the initial purification, a
175 Waters 19 × 300 mm symmetry prep C8 reverse phase column was used. The mobile phases
176 consisted of 100% acetonitrile (CH_3CN) and 0.5% of acetic acid in water (v/v). For the initial
177 separation, a gradient of 5% CH_3CN to 95% CH_3CN over 35 minutes was used with a flow rate
178 of 8 mL/min. Ten fractions (volume 40 mL each) at 5 min intervals were collected and tested for
179 COX-2 inhibitory activity. Fraction eight (F8; yield: 360 mg) had the highest activity and was
180 further purified using similar conditions as before except the gradient was run over 70 min. Four
181 fractions were collected from F8 and they were subjected to the COX-2 assay. The most active

182 fraction, F8-2 (4mL volume, eluted at 59.2 min. at 282 nm; yield 90 mg), was then purified using
183 a 10 × 250 mm Curacil-PFP column and an isocratic run with a mobile phase consisting of 0.5%
184 acetic acid in water:methanol:CH_3CN (2:3:5) at flow rate 3 mL/min. Four fractions were
185 collected from F8-2 and F8-2-3 (1.45 mL volume, eluted at 8.67 min., at 282 nm; yield 49.5 mg)
186 was found to have the highest inhibitory activity on COX-2 enzyme. This fraction was re-
187 fractionated using the conditions described previously, with the exception of the flow rate which
188 was reduced to 1 mL/min. Three fractions were collected from F8-2-3 and F8-2-3-2 (1.48 mL
189 volume, eluted at 25.29 min., at 282 nm; yield: 32.2 mg) had the highest inhibitory activity. This
190 fraction was identified as [6]-paradol (95% purity) using LC-MS and ^{1}H, ^{13}C and 2D-NMR
191 data[20, 21]. The purification procedure was repeated multiple times to obtain sufficient amounts of
192 [6]-paradol for *in vitro* and *in vivo* experiments.
193 Chemical analysis of the extract revealed three major peaks (Fig.1). The UV spectra and mass
194 fragmentation patterns of these peaks matched the compounds of the gingerol family previously
195 identified in *Aframomum melegueta*[22] and in other members of Zingiberaceae[23]. The most
196 abundant were putatively identified as [6]-gingerol, [6]-shogaol and [6]-paradol or 1.0 mg/g [6]-
197 gingerol, 0.53 mg/g [6]- shogaol and 1.2 mg/g [6]-paradol per gram of the extract. All three
198 compounds were assayed for COX-2 enzyme inhibitory activity at the same concentration
199 (1mg/mL) as the whole extract and Vioxx®. [6]-paradol was the most active with 91% inhibition,
200 followed by [6]-shogaol (68%), and [6]-gingerol (7%) (Table1). Paradol inhibitory activity was
201 higher than Vioxx® (87%) and the whole extract (76%) confirming to be the most active
202 compound as found in bioactivity guided fractionation assay. [6]-Gingerol and [6]-shogaol were
203 commercially purchased and [6]-paradol was isolated by bioactivity guided fractionation (as
204 described above).

205

206 Inhibition of expression of pro-inflammatory genes (*in vitro*) - in addition to inhibiting pro-
207 inflammatory COX-2 enzyme, the extract of grains of paradise was also tested for inhibition of
208 pro-inflammatory genes. The genes tested were tumor necrosis factor alpha (TNFa), interleukin-
209 1beta (IL-1b), interleukin-6 (IL-6), COX-2 and inducible nitric oxide synthase (iNOS)
210 The *in vitro* experiments were designed to quantify the relative amount of transcripts for target
211 genes (COX2, iNOS, TNFα, IL6 and IL1β) within the total RNA in individual cell batches
212 following treatments with the extract or its three major gingerols. All experiments were started
213 with an equal number of cells for each treatment. For each assay, two control sets were
214 monitored (Fig. 2). The *in vitro* experiments showed that both ethanolic extract of grains of
215 paradise extract and [6]-shogaol were inhibitory in a dose dependent manner ($p < 0.05$ by
216 ANOVA) on IL1β (Fig. 2) but not on other genes tested.. The extract was significantly cytotoxic
217 to macrophages at concentration higher than 20µg/mL and hence could not be tested for gene
218 expression inhibition beyond that concentration. [6]-shogaol did not affect the transcription of
219 other genes at 5 µg/mL, its highest non-toxic concentration. [6]-paradol and [6]-gingerol did not
220 show any significant inhibitory activities at the non-cytotoxic concentration range up to 30 and
221 50 µg/mL respectively (data not shown).
222 Anti-inflammatory activity in rat paw edema assay (*in vivo*) - to investigate whether the observed
223 *in vitro* COX-2 enzyme and gene expression inhibitory properties translate into *in vivo* anti-
224 inflammatory activity, we tested the extract in a rat paw carrageenan edema assay, which is
225 commonly used for testing anti-inflammatory agents. Initially, two doses of the whole extract
226 were given p.o. (orally) to the animals – 1000 and 500 mg/kg. Aspirin was used as a positive
227 control at 150 mg/kg dose. At 1000 mg/kg, the extract demonstrated anti-inflammatory effect,

228 reducing edema by 49% (Table3). At the lower dose (500 mg/kg) the extract exhibited a reduced
229 anti-inflammatory effect (11% edema reduction). At the same time, aspirin reduced edema by
230 43% at 150 mg/kg. Therefore, grains of paradise extract does have *in vivo* anti-inflammatory
231 activity, but it was not as effective as aspirin.
232 To analyze anti-inflammatory activity of single compounds, the most active compound in the
233 COX-2 enzymatic assay, [6]-paradol, has been isolated from the extract and tested in *in vivo*
234 assay at the same dose level as aspirin (150 mg/kg) which was used as a positive control. At this
235 dose it inhibited the rat paw edema by 20% and aspirin by 36%, respectively. Two other
236 compounds from the extract, [6]-gingerol and [6]-shogaol, given at 150 mg/kg, inhibited edema
237 by 25% and by 38% respectively. In the same assay aspirin given at 150 mg/kg inhibited the
238 edema by 37%. Therefore, the [6]-shogaol, the most active compound in the gene expression
239 assays, was also the most active compound *in vivo* and demonstrated anti-inflammatory activity
240 similar to that of aspirin (Table3).
241
242 Results of our study demonstrated the inhibitory activity of grains of paradise ethanolic extract in
243 COX-2 *in vitro* enzymatic assay. The bioactivity guided fractionation revealed that the [6]-
244 paradol present in the extract is the most effective inhibitor of COX-2 enzymatic activity. [6]-
245 paradol and two other major compounds present in the extract, [6]-gingerol and [6]-shogaol,
246 belong to the gingerol family of compounds. The results for the enzymatic inhibition support
247 previous reports on gingerols as inhibitors of the arachidonic acid biosynthetic pathway. In one
248 study, ginger rhizome extract (*Zingiber officinale*, Roscoe, Zingiberaceae) was investigated for
249 inhibition of COX-1 enzyme and anti-platelet aggregation activity. [8]-Paradol, a natural
250 constituent of ginger was the most active inhibitor and stronger than aspirin[24]. Similar studies on

COX-2 enzyme inhibitory activity revealed that ginger constituents, [8]-paradol and [8]-shogaol, had strong inhibitory activity[25]. Additionally, structure activity study on gingerols and COX-2 inhibition has shown that the paradols showed the highest affinity for COX-2 followed by the shogaols and then the gingerols[26]. Our data, together with the above mentioned studies on compounds from ginger, suggest that compounds from the gingerol family are cyclooxygenase inhibitors. When the gingerols from the extract were tested for the inhibition of expression of proinflammatory genes (COX2, iNOS, TNFα, IL6 and IL1β) the whole extract and [6]-shogaol exhibited the inhibitory activity on IL1β gene expression while other genes have not been affected.

To evaluate how these *in vitro* activities at enzyme and gene level transpond into *in vivo* activity we used the cyclooxygenase-dependent carrageenan induced paw edema assay[27],a standard model to reproduce acute inflammation *in vivo.* The ethanolic extract of grains of paradise exhibited anti-inflammatory activity in this assay. This also confirmed the anti-inflammatory activity reported earlier for aqueous extract of grains of paradise[5, 12]. Additionally, the three major gingerols present in the extract were also effective in reducing the inflammation with [6]-shogaol at the level comparable to the aspirin.

Since grains of paradise ethanolic extract and major gingerols present in it, showed *in vitro* inhibition of COX-2 enzyme activity and ethanolic extract and [6]-shogaol reduction in expression of pro-inflammatory IL1β gene, we could hypothesize that the extract may have a dual mode of action, at the gene and the enzyme level. Inhibition by [6]-paradol and other gingerols present in the extract of COX-2 enzyme activity, and inhibition by [6]-shogaol of pro-inflammatory gene expression, could impair prostaglandin, (the end product of cyclooxygenase pathway) production thereby reducing the inflammatory process *in vivo.* Gingerols from ginger

(*Zingiber officinale*) have been previously reported to inhibit prostaglandin biosynthesis[28]. However, to put more light on this, our extract and its compounds require further studies; especially how the whole ethanolic extract and its three major isolated gingerols affect prostaglandin levels. In conclusion, this study with *in vitro* and *in vivo assays* has demonstrated that the ethanolic extract of grains of paradise and its major compounds have an anti-inflammatory potential at the enzyme and *in vivo* level, but they need to be further evaluated.

ACKNOWLEDGEMENTS

Excellent technical assistance was provided by Reneta Pouleva and Ruth Dorn. We thank Cheryl Lyn Dybas for critical reading of the manuscript.

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Funding

Research supported by Phytomedics Inc. (Jamesburg NJ, USA); NIH Center for Dietary Supplements Research on Botanicals and Metabolic Syndrome, grant # 1-P50 AT002776-01; Fogarty International Center of the NIH under U01 TW006674 for the International Cooperative Biodiversity Groups; and Rutgers University. Additional support came from National Center for Complimentary and Alternative Medicine of NIH under 1-K99 AT004245-01.

FIGURE LEGENDS

Figure 1. HPLC chromatogram of Grains of Paradise (*A. melegueta*) extract showing putative compounds; 1: [6]-gingerol; 2: [6]-shogaol 3: [6]-paradol.

Figure 2. Effect of grains of paradise extract (A) and [6]-shogaol (B) on IL1β gene expression in LPS-activated RAW macrophages measured by the mRNA quantity relative to the response to LPS activation only (positive control) that is normalized to a value of 1.00; Lower values represent greater inhibitory effects with 0.00 corresponding to a complete inhibition of the induced gene expression; Values are mean ± S.D.. *: $p < 0.05$ (compared with positive control)

Table 1. *In vitro* COX-2 Enzyme Inhibition By The Ethanolic Extract Of Grains Of Paradise And Its Anti-inflammatory Components.[a]

Treatment	**Inhibition (%)**±SD
control[b]	2 ± 2
A. melegueta extract	76 ± 6.7***
[6]-gingerol	7 ± 11.7
[6]-shogaol	68 ± 6.4***
[6]-paradol	91 ± 3.6***
Vioxx®	87 ± 1.5***

[a]Data are shown as mean inhibition ±SD in %. Significant levels are: ***: 0.1%.

[b]All samples were tested at the concentration of 1 mg/mL. Control – celery extract at 1 mg/mL, whole extract – Grains of Paradise (*A. melegueta*) extract at 1 mg/mL, Vioxx® (MW 314.36) at 1 mg/mL – 3.18 mM, [6]-gingerol (MW 294.38) at 1mg/mL – 3.39 mM, [6]-shogaol (MW 276.37) at 1mg/mL - 3. 62 mM, [6]-paradol (MW 278) at 1 mg/mL - 3.60 mM.

Table 2. Primer Sequences Used For RT-PCR.

Gene (accession number)	Forward	Reverse
COX-2 (NM_011198)	5'-TGGTGCCTGGTCTGATGATG-3'	5'-GTGGTAACCGCTCAGGTGTTG-3'
iNos2 (XM_147149)	5'-CCCTCCTGATCTTGTGTTGGA-3	5'-TCAACCCGAGCTCCTGGAA-3'
IL1□(NM_008361)	5'-CAACCA ACAAGTGATATTCTCCATG -3'	5'-GATCCACACTCTCCA GCTGCA-3'
TNF-α (NM_013693)	5'-CATCTTCTCAAAATTCGAGTGACAA-3'	5'-TGGGAGTAGACAAGGTACAACCC-3'
IL6 (NM_031168)	5' TAGTCCTTCCTACCCCAATTTCC-3'	5' TTGGTCCTTAGCCACTCCTTC-3'
Actin (NM_007393)	5'-AACCGTGAAAAGATGACCCAGAT-3'	5'-CACAGCCTGGATGGCTACGT-3'

Table 3. Inhibition Of Carrageenan Induced Rat Paw Edema By Grains Of Paradise Ethanolic Extract And Its Main Components [6]-gingerol, [6]-shogaol, and [6]-paradol With Aspirin As A Positive Control.[a]

Treatment	**Dose**	**Average Paw difference ± SD**	**Inhibition (%)**
I			
Vehicle	10 mL/kg	90 ± 3.5	--
A. melegueta extract	500 mg/kg	80 ± 13.7	11
	1000 mg/kg	46 ± 4.2**	49
Aspirin	150 mg/kg	51 ± 5.1**	43
II			
Vehicle	10 mL/kg	85 ± 3.3	--
[6]-paradol	150 mg/kg	68 ± 6.7*	20
Aspirin	150 mg/kg	54 ± 8.5***	36
III			
Vehicle	10 mL/kg	79 ± 3.4	--
[6]-shogaol	150 mg/kg	49 ± 5.4***	38
[6]-gingerol	150 mg/kg	59 ± 8.5**	25
Aspirin	150 mg/kg	50 ± 8.6***	37

[a]Test substances were administered orally 60 min before the right hind paw received injection of carrageenan (0.1 mL of 1% suspension intraplantar). Hind paw edema was measured 3 h later; size of the paws was compared, average paw difference (expressed in mL) established and inhibition was calculated relative to the vehicle treated animals in the control group. Data are shown as mean ± SD and percentages of inhibition. Significant levels are: *: 5%, **: 1%, ***: 0.1%.

Figure 1.

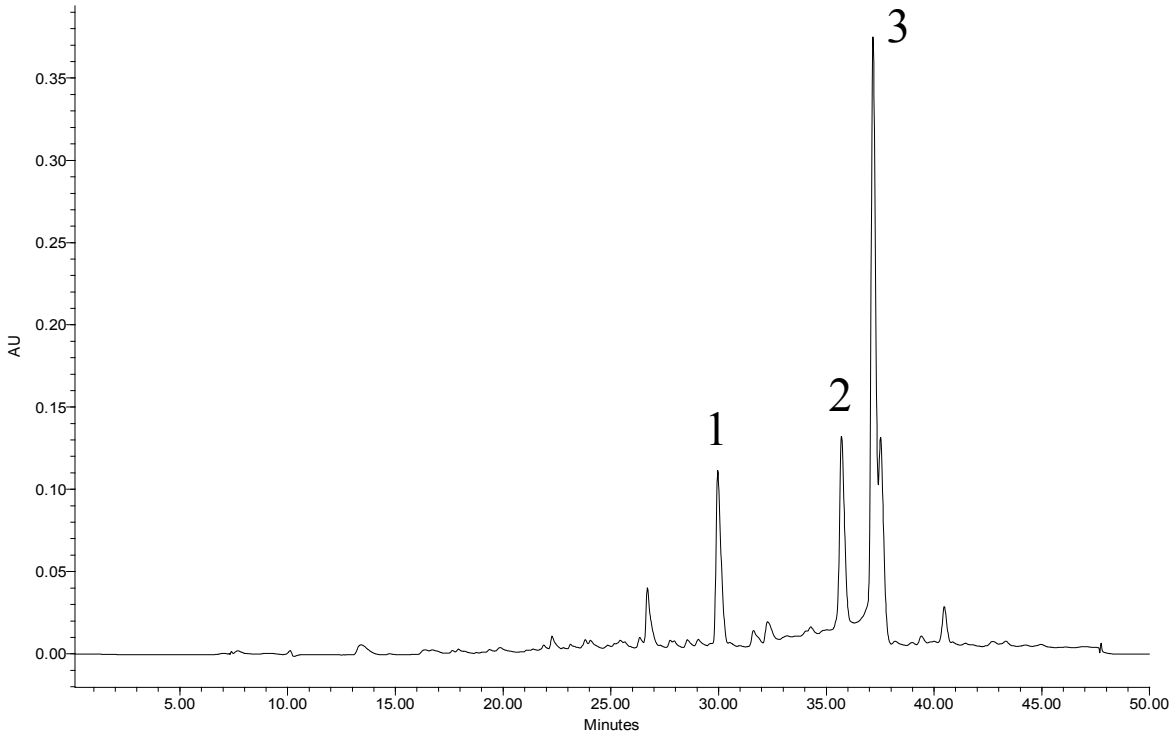
3
2
1
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
AU
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Minutes

Figure 2.

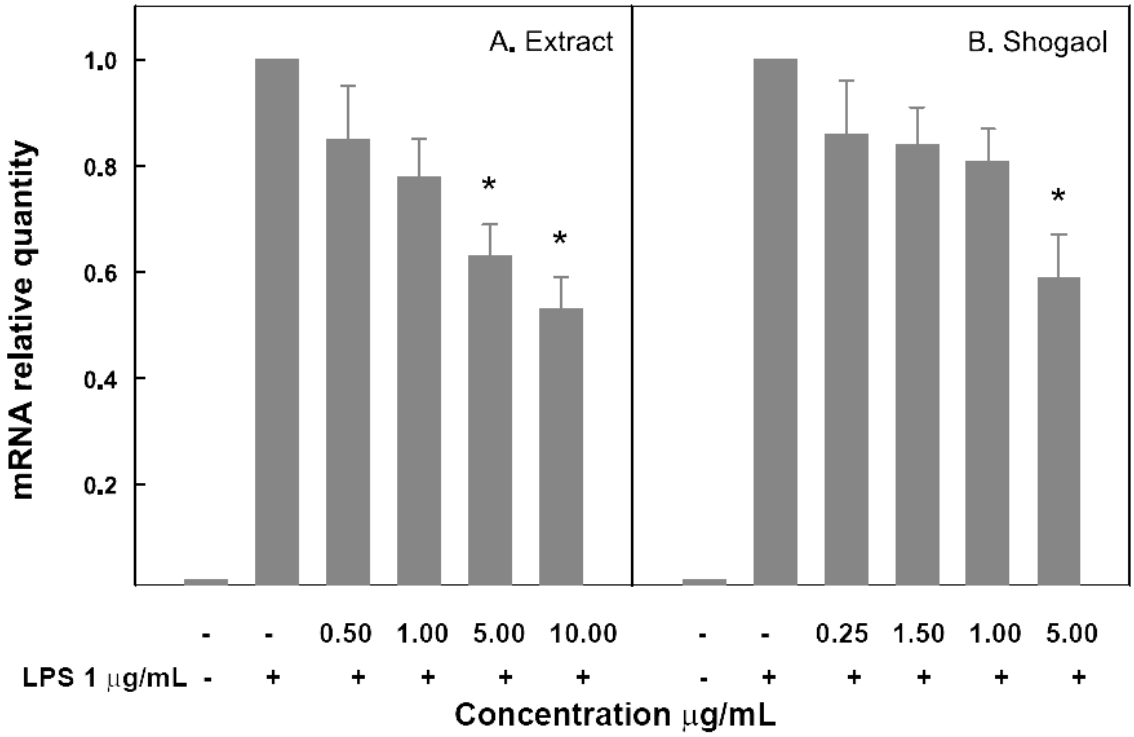
A. Extract
B. Shogaol
mRNA relative quantity
1.0
0.8
0.6
0.4
0.2
*
*
*
- - 0.50 1.00 5.00 10.00 - - 0.25 1.50 1.00 5.00
LPS 1 μg/mL - + + + + + - + + + + +
Concentration μg/mL

FIGURE CAPTIONS

Figure 2.

A. Extract (in the left graph)

B. Shogaol (in the graphon the right)

Graphic abstract