

Anti-inflammatory and Anti-oxidant Properties of *Curcuma longa* (Turmeric) Versus *Zingiber officinale* (Ginger) Rhizomes in Rat Adjuvant-Induced Arthritis

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Abstract—Turmeric (rich in curcuminoids) and ginger (rich in gingerols and shogaols) rhizomes have been widely used as dietary spices and to treat different diseases in Ayurveda/Chinese medicine since antiquity. Here, we compared the anti-inflammatory/anti-oxidant activity of these two plants in rat adjuvant-induced arthritis (AIA). Both plants (at dose 200 mg/kg body weight) significantly suppressed (but with different degrees) the incidence and severity of arthritis by increasing/decreasing the production of anti-inflammatory/pro-inflammatory cytokines, respectively, and activating the anti-oxidant defence system. The anti-arthritic activity of turmeric exceeded that of ginger and indomethacin (a non-steroidal anti-inflammatory drug), especially when the treatment started from the day of arthritis induction. The percentage of disease recovery was 4.6–8.3% and 10.2% more in turmeric compared with ginger and indomethacin ($P < 0.05$), respectively. The present study proves the anti-inflammatory/anti-oxidant activity of turmeric over ginger and indomethacin, which may have beneficial effects against rheumatoid arthritis onset/progression as shown in AIA rat model.

KEY WORDS: rat adjuvant-induced arthritis; anti-inflammatory/anti-oxidant activity; *Curcuma longa*; indomethacin; *Zingiber officinale*.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease that affects 1% of the adult population worldwide [1]. It leads to significant disability and a consequent reduction in quality of life, which have a substantial socio-economic impact [2]. Tumour necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6 are known to be the primary cytokines that mediate the marked destruction of cartilage/bone in arthritis, and have thus been investigated as important biological targets to develop drugs for the treatment of active RA [3, 4]. These pro-inflammatory cytokines induce fever,

wasting, and a number of inflammatory events underlying RA including leucocytes adhesion/recruitment, angiogenesis, and tissue destruction/fibrosis [5]. In addition, they trigger the synthesis of proteolytic enzymes such as matrix metalloproteinases (MMPs) and the formation of osteoclasts, which ultimately lead to the destruction of joints and the impairment of their function [6]. The free radicals especially reactive oxygen species (ROS) are also involved in the pathogenesis of RA and induce cartilage destruction through either a direct degradative effect on matrix components or an indirect action via activation of MMPs [7]. Rat adjuvant-induced arthritis (AIA) shares several features with human RA including weight loss, oxidative tissue damage and inflammatory infiltration of synovial membrane in association with joints swelling/destruction [5, 8]. In this model, arthritis develops within 14–18 days after adjuvant injection [5] through cell-mediated autoimmunity by structural mimicry between the mycobacterium capsule and the cartilage proteoglycans [9].

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Presently, many steroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs, immunosuppressants and anti-cytokines are used to control inflammatory symptoms/pain in RA patients [10]. The major concerns with these drugs are poor efficacy with chronic use, potential toxic effects, gastrointestinal/cardiovascular disorders, immunodeficiency, and high costs [11–13]. Therefore, scientists have been paying serious attention to herbal therapies that have anti-inflammatory/anti-oxidant activity and minimum side effects to treat RA. Recently, the use of botanical remedies for arthritis treatment is promoted in USA, especially after the withdrawal of FDA-approved anti-inflammatory drugs [14]. *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) are medicinal plants belonging to Zingiberaceae family and cultivated in Asia. Their rhizomes have been widely used as dietary spices and to treat different diseases in Ayurveda/Chinese medicine since antiquity. Some studies reported the anti-arthritic activity of extracts/components of these plants [14–20]. However, whether the anti-arthritic activity of turmeric rhizomes is more or less potent than ginger rhizomes has not been established. Therefore, the present study aimed to evaluate and compare the prophylaxis and therapeutic abilities of the rhizomes powder of these plants in AIA rat model (from the day of arthritis induction and onset, respectively), especially with reference to the anti-inflammatory and anti-oxidant properties. Indomethacin (one of NSAIDs) was used as a reference drug in this study. Because turmeric and ginger rhizomes are used as dietary spices, they were given to animals via oral administration. Furthermore, the current study examined any deleterious/toxic effects caused by consuming the rhizomes of these plants.

MATERIALS AND METHODS

Materials

Complete Freund's adjuvant (CFA) containing 1.0 mg of dry, heat-killed *Mycobacterium tuberculosis* (strain H37Ra) per 1.0 ml sterile, non-metabolizable oils (0.85 ml paraffin oil and 0.15 ml of mannide mono-oleate) was purchased from Sigma-Aldrich (St Louis, MO, USA). Indomethacin ($C_{19}H_{16}ClNO_4$; molecular weight, 357.79 Da) powder was purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA, USA). Pure (100%) *C. longa* and *Z. officinale* rhizomes powder (AL-AMEER Brand) were authenticated and

purchased from a herbal-specialised company (Kazer-ooni Brothers Est. Co., Manama, Bahrain). Adult male Wistar albino rats (*Rattus norvegicus*), weighing ~120–130 g, were obtained from College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Hufuf, Kingdom of Saudi Arabia. Animals were housed in suitable cages and acclimatised to laboratory conditions for a period of 1 week before the commencement of the experiments. Rats were fed standard rodent food pellets (ARASCO, Riyadh, Kingdom of Saudi Arabia) and distilled water. All animals were humanely treated in accordance with WHO guideline for animal care and the study design was approved by the King Faisal University Research Ethics Committee. Once arthritis developed, food was served on the bottom of the cages as severely arthritic rats have difficulty in feeding from the cage top.

Experimental Design and Treatment Schedule

Animals were randomly divided into nine groups of six animals each: six arthritis groups and three healthy groups. Arthritis was induced by a single intra-dermal injection of 0.1 ml of CFA into the palmar surface of the left hind paw after the rats were subjected to light diethyl ether anaesthesia [21]. Arthritic rats received orally (by gavage) and daily (for 28 consecutive days) 1.0 ml distilled water as vehicle (arthritis control group), indomethacin (1.0 mg/kg body weight), or 200 mg/kg body weight of either turmeric or ginger rhizomes powder suspended in 1.0 ml distilled water (TRPS or GRPS, respectively) from the day of arthritis induction (day 0). Other arthritic rats received either TRPS or GRPS for 14 consecutive days started from the day of arthritis onset (day 15). Rats in the healthy groups were subjected to light diethyl ether anaesthesia, as in the arthritis groups, and injected with a single dose of 0.1 ml of physiological saline and received orally and daily (for 28 consecutive days) 1.0 ml distilled water (healthy control group), TRPS or GRPS.

Blood and Tissue Sampling

Animals were fasted overnight and subjected to light diethyl ether anaesthesia before killing on day 29. The blood was collected into clean test-tubes with or without EDTA. A portion of blood with EDTA was used to measure the total and differential leucocyte counts by automated haematology analyzer (CELL-DYN 3700, Abbott Laboratories, Abbott Park, Illinois, USA). Another blood portion without EDTA was used to

separate serum, which was divided into aliquots and preserved at -80°C for further analysis. Thymus and spleen were immediately removed, cleaned and weighed. Bone marrow was also collected from the left femur bone. The liver was quickly perfused *in situ* (via the hepatic portal vein) with ice-cold saline (to remove erythrocytes and clots) and homogenised in cold buffer after the gall bladder was dissected away. The homogenate was collected and its protein content was assayed by the method of Lowry *et al.* [22]. Thereafter, it was divided into aliquots and stored at -80°C until used for the determination of tissue lipid peroxides (LPO) and anti-oxidants.

Measurements

Hind paws oedema and the body weight gain were periodically measured every 3–4 days after arthritis induction using a plethysmometer (Model: 520R, IITC Life Science Inc., Woodland Hills, CA, USA) and a 0.1 g precision balance (Model: Adventurer Pro AV4101, Ohaus Corporation, Pine Brook, NJ, USA), respectively. Food intake (on a per-group basis) was measured weekly. The percentage of the disease recovery was evaluated by measuring the percentage inhibition of hind paws oedema on day 29 using the following equation: $(V_c - V_t/V_c) \times 100$ [23], where V_c is the mean value of hind paws oedema in arthritis group that received vehicle and V_t is the mean value of hind paws oedema in arthritis groups that received treatment (indomethacin, TRPS or GRPS). The cellularity of lymphoid organs (bone marrow, thymus and spleen) was measured by a Neubauer counting chamber (Paul Marienfeld GmbH, Bad Mergentheim, Germany) after lysing the erythrocytes by RBC lysis solution (AppliChem GmbH, Darmstadt, Germany). Levels of serum pro-inflammatory and anti-inflammatory cytokines were measured using commercially available ELISA kits for TNF- α , IL-1 β , IL-4, IL-6, and IL-10 (Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer's recommendations.

Serum alanine aminotransferase (ALAT) activity was determined using a coupled reaction where the serum dependent absorbance change of NADH oxidation at 340 nm was monitored in the presence of optimised concentrations of L-alanine, α -ketoglutarate and purified lactic dehydrogenase enzyme [24]. Serum alkaline phosphatase (ALP) activity was determined from the rate of conversion of *p*-nitrophenylphosphate to *p*-nitrophenol [25]. Malondialdehyde (MDA) was measured through estimation of a stable chromophore

formed from the reaction of a chromogenic reagent, *N*-methyl-2-phenylindole, with MDA at 45°C [26]. Catalase (CAT) activity was measured from the rate of dismutation of H_2O_2 to water and molecular oxygen in a two-step coupling reaction [27]. Glutathione peroxidase (GPx; selenium-dependent) activity was determined from the azide insensitive rate of oxidation of NADPH in the presence of H_2O_2 , glutathione, and glutathione reductase (GR) [28]. GR activity was measured from the rate of reduction of oxidised glutathione by NADPH [29]. Superoxide dismutase (SOD) activity was measured in a coupling reaction through formation of formazan dye from tetrazolium salt by superoxide radicals generated by xanthine oxidase and hypoxanthine [30]. Reduced glutathione (GSH) was determined from the reaction of sulfhydryl group with Ellman's reagent to give 5-thio-2-nitrobenzoic acid [31].

Statistics

Data are presented as mean value \pm SEM. Statistical analysis was performed with ANOVA and the differences among groups were determined by Bonferroni's multiple comparison test [32] using GraphPad Prism version 4.03 for Windows (GraphPad software Inc., San Diego, California, USA). *P* values of <0.05 , <0.01 and <0.001 were considered statistically significant, highly significant and very highly significant, respectively.

RESULTS

Modulatory Effects of Turmeric *Versus* Ginger Rhizomes on Hind Paws Oedema and Body Weight Loss of Arthritic Rats

Swelling and redness developed over a 24-h period in the hind paw injected with CFA and reached maximum intensity on day 4 (first swelling phase). Thereafter, swelling slowly subsided until the eighth day and then the paw began to swell again when disseminated arthritis appeared (second swelling phase, which was greater than the first one and peaked on days 22–25). During the second swelling phase, the non-inject hind paw also began to swell. Oral administration of TRPS or GRPS either from the day of arthritis induction (day 0) or onset (day 15) as well as indomethacin significantly suppressed ($P < 0.01$ to $P < 0.001$) the second swelling phase, which occurs at the appearance of polyarthritis, and decreased the hind paws oedema/redness in the

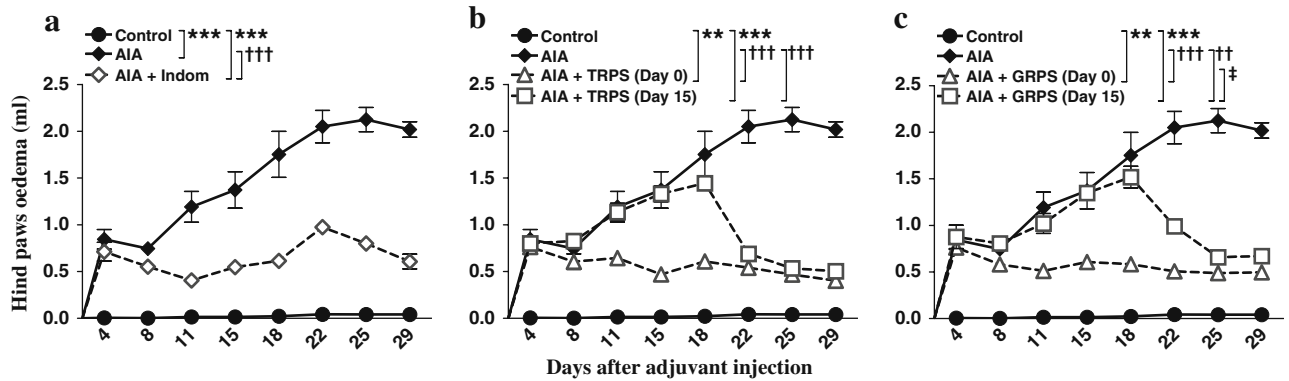


Fig. 1. The modulatory effects of indomethacin (a), turmeric (b) and ginger (c) on hind paws oedema of arthritic rats. Hind paws oedema=mean volume of both hind paws on days from 4 to 29 after arthritis induction—mean volume of both hind paws on day 0 before arthritis induction. SEM represented by vertical bars. *AIA* rat adjuvant-induced arthritis, *Indom* indomethacin, *TRPS* turmeric rhizomes powder suspension, *GRPS* ginger rhizomes powder suspension. ** $P < 0.01$; *** $P < 0.001$ (versus the healthy control group). ††† $P < 0.001$; †††† $P < 0.0001$ (versus the arthritis control group, which received vehicle). ‡ $P < 0.05$ (versus the arthritis group, which received rhizomes powder from the day of arthritis induction).

arthritis groups (Fig. 1). The percentages of disease recovery (as measured by the percentage inhibition of hind paws oedema on day 29) in arthritis groups that received indomethacin, TRPS or GRPS from day 0, and TRPS or GRPS from day 15 were 69.9%, 80.1%, 75.5%, 75.1%, and 66.8%, respectively, indicating that TRPS was more effective than GRPS and indomethacin ($P < 0.05$).

As shown in Fig. 2a, arthritic rats that received vehicle gained less weight especially in the first 2 weeks after arthritis induction compared with the healthy control animals ($P < 0.001$). This markedly loss of body weight gain was partially improved by indomethacin ($P < 0.05/P < 0.001$ compared with the healthy/arthritis

control groups, respectively). Oral administration of TRPS or GRPS from day 0 completely and equally modulated the loss of body weight gain in the arthritic rats ($P > 0.05/P < 0.001$ compared with the healthy/arthritis control groups, respectively). This modulatory effect of TRPS or GRPS was weak and insignificant ($P > 0.05$ compared with the arthritis control group) when the treatment started from day 15 (Fig. 2b–c). The loss of body weight gain in arthritic rats that received vehicle and its complete modulation by either TRPS or GRPS from day 0 were positively correlated ($P < 0.05$, Pearson’s rank correlation) with the decrease ($r_s = 0.97$) and increase ($r_s = 0.95$ and 0.92) in food intake, respectively (data not shown).

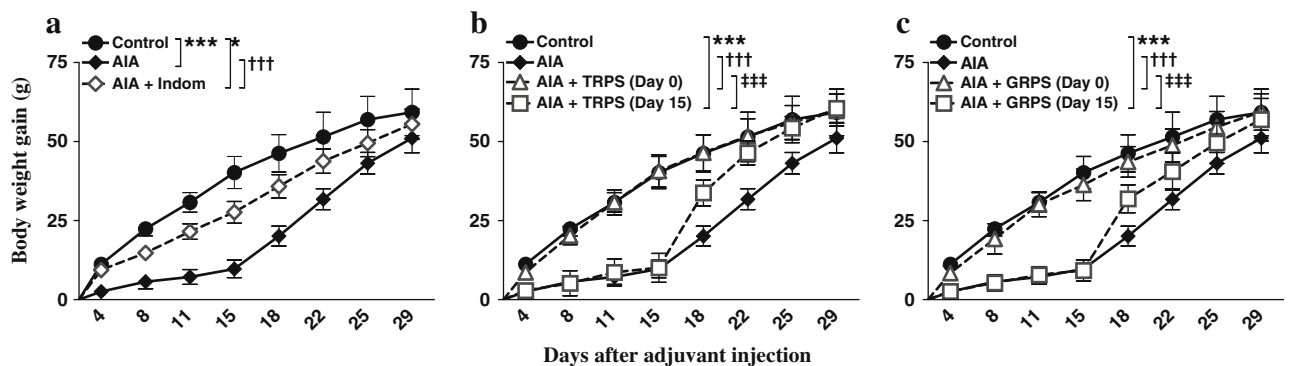


Fig. 2. The modulatory effects of indomethacin (a), turmeric (b) and ginger (c) on the decrease in body weight gain of arthritic rats. Body weight gain=body weight on days from 4 to 29 after arthritis induction—body weight on day 0 before arthritis induction. SEM represented by vertical bars. *AIA* rat adjuvant-induced arthritis, *Indom* indomethacin, *TRPS* turmeric rhizomes powder suspension, *GRPS* ginger rhizomes powder suspension. * $P < 0.05$; *** $P < 0.001$ (versus the healthy control group). †††† $P < 0.0001$ (versus the arthritis control group, which received vehicle). ††††† $P < 0.00001$ (versus the arthritis group, which received rhizomes powder from the day of arthritis induction).

Modulatory Effects of Turmeric *Versus* Ginger Rhizomes on the Changes in Weights and Cellularity of Lymphoid Organs of Arthritic Rats

The relative weight and cellularity of spleen as well as the bone marrow cellularity significantly increased ($P < 0.05$ to $P < 0.01$), but the thymic relative weight and cellularity significantly decreased ($P < 0.05$), in arthritic rats that received vehicle compared with the healthy control animals (Fig. 3). These changes were reverted to near normal levels upon treatment with TRPS, GRPS or indomethacin ($P > 0.05$ compared with the healthy control group), but the utmost modulation on the changes in lymphoid organs was shown in arthritis groups that received TRPS, especially from day 0 (Fig. 3).

Modulatory Effects of Turmeric *Versus* Ginger Rhizomes on the Changes in Total and Differential Leucocyte Counts of Arthritic Rats

Table 1 revealed that the significant increase in blood total leucocyte, granulocyte and agranulocyte counts ($P < 0.01$ to $P < 0.001$) shown in arthritic rats that received vehicle compared with the healthy control animals were mainly due to neutrocytosis ($P < 0.05$) and lymphocytosis ($P < 0.001$). These changes were reverted to near normal levels upon treatment with TRPS, GRPS or indomethacin ($P > 0.05$ compared with the healthy control group), but the utmost modulation on

the changes in blood leucocytes was shown in arthritis groups that received TRPS and GRPS from day 0 (Table 1).

Modulatory Effects of Turmeric *Versus* Ginger Rhizomes on the Changes in Pro-Inflammatory and Anti-Inflammatory Cytokines of Arthritic Rats

Levels of the pro-inflammatory (TNF- α , IL-1 β , and IL-6; Fig. 4a-c) and the anti-inflammatory (IL-4 and IL-10; Fig. 4d, e) cytokines in serum of arthritic rats that received vehicle significantly increased ($P < 0.001$) and decreased ($P < 0.05$), respectively, compared with the healthy control animals. Indomethacin completely modulated the increase in IL-6 ($P > 0.05/P < 0.001$), partially alleviated the increase in TNF- α and IL-1 β ($P < 0.05$ to $P < 0.01/P < 0.05$), and did not show any modulation on the decrease in the anti-inflammatory cytokines ($P < 0.05/P > 0.05$) of arthritic rats compared with the healthy/arthritis control animals, respectively (Fig. 4). Oral administration of GRPS from either days 0 or 15 completely modulated all the changes shown in serum IL-6 ($P > 0.05/P < 0.001$) and anti-inflammatory cytokines ($P > 0.05/P < 0.05$), but partially alleviated the increase in serum TNF- α and IL-1 β ($P < 0.05/P < 0.01$), of arthritic rats compared with the healthy/arthritis control animals, respectively (Fig. 4). The changes in pro-inflammatory and anti-inflammatory cytokines of arthritic rats were completely modulated only in arthritic

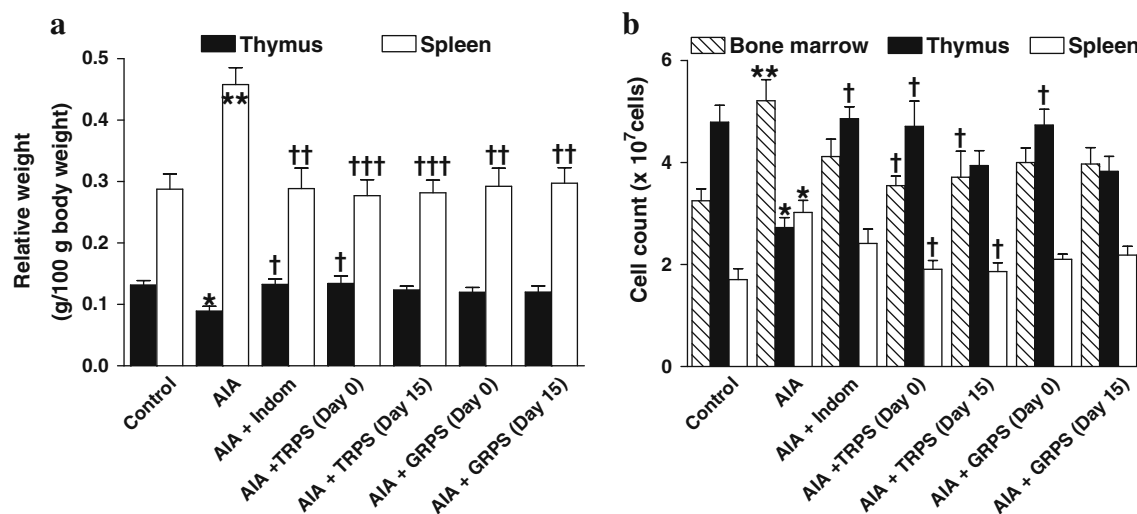


Fig. 3. The modulatory effects of indomethacin, turmeric and ginger on the changes in weights (a) and cellularity (b) of lymphoid organs of arthritic rats. SEM represented by vertical bars. AIA rat adjuvant-induced arthritis, Indom indomethacin, TRPS turmeric rhizomes powder suspension, GRPS ginger rhizomes powder suspension. * $P < 0.05$; ** $P < 0.01$ (versus the healthy control group). † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$ (versus the arthritis control group, which received vehicle).

Table 1. The Modulatory Effects of Indomethacin, Turmeric and Ginger on the Changes in Total and Differential Leucocyte Counts (10^3 cells/mm³) of Arthritic Rats

	AIA ⁺						
	Control	AIA	Indom	TRPS		GRPS	
				Day 0	Day 15	Day 0	Day 15
Total leucocytes	7.93±0.54	18.43±1.19***	12.01±1.31†††	9.31±0.76†††	10.60±1.13†††	9.61±0.87†††	11.23±1.14†††
Total granulocytes	2.14±0.27	5.57±1.19**	3.26±1.05	3.05±0.17	2.89±0.30	2.99±0.19	2.95±0.65
Basophils	0.53±0.22	0.70±0.25	0.54±0.26	0.07±0.02	0.24±0.13	0.22±0.13	0.25±0.13
Eosinophils	0.46±0.04	0.54±0.14	0.57±0.08	0.52±0.06	0.38±0.06	0.43±0.08	0.42±0.03
Neutrophils	1.15±0.40	4.34±1.32*	2.15±0.77	2.47±0.20	2.26±0.24	2.34±0.22	2.28±0.53
Total agranulocytes	5.80±0.75	12.86±1.74***	8.76±1.16	6.25±0.75†††	7.72±1.03†	6.61±0.77††	8.28±0.76
Monocytes	0.38±0.17	0.54±0.28	0.26±0.13	0.04±0.01	0.23±0.18	0.16±0.12	0.23±0.18
Lymphocytes	5.42±0.80	12.31±1.65***	8.49±1.22	6.21±0.75††	7.50±1.00†	6.45±0.83††	8.06±0.71

Values are means±SEM

AIA rat adjuvant-induced arthritis, Indom indomethacin, TRPS turmeric rhizomes powder suspension, GRPS ginger rhizomes powder suspension
 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (versus the healthy control group); † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$ (versus the arthritis control group, which received vehicle)

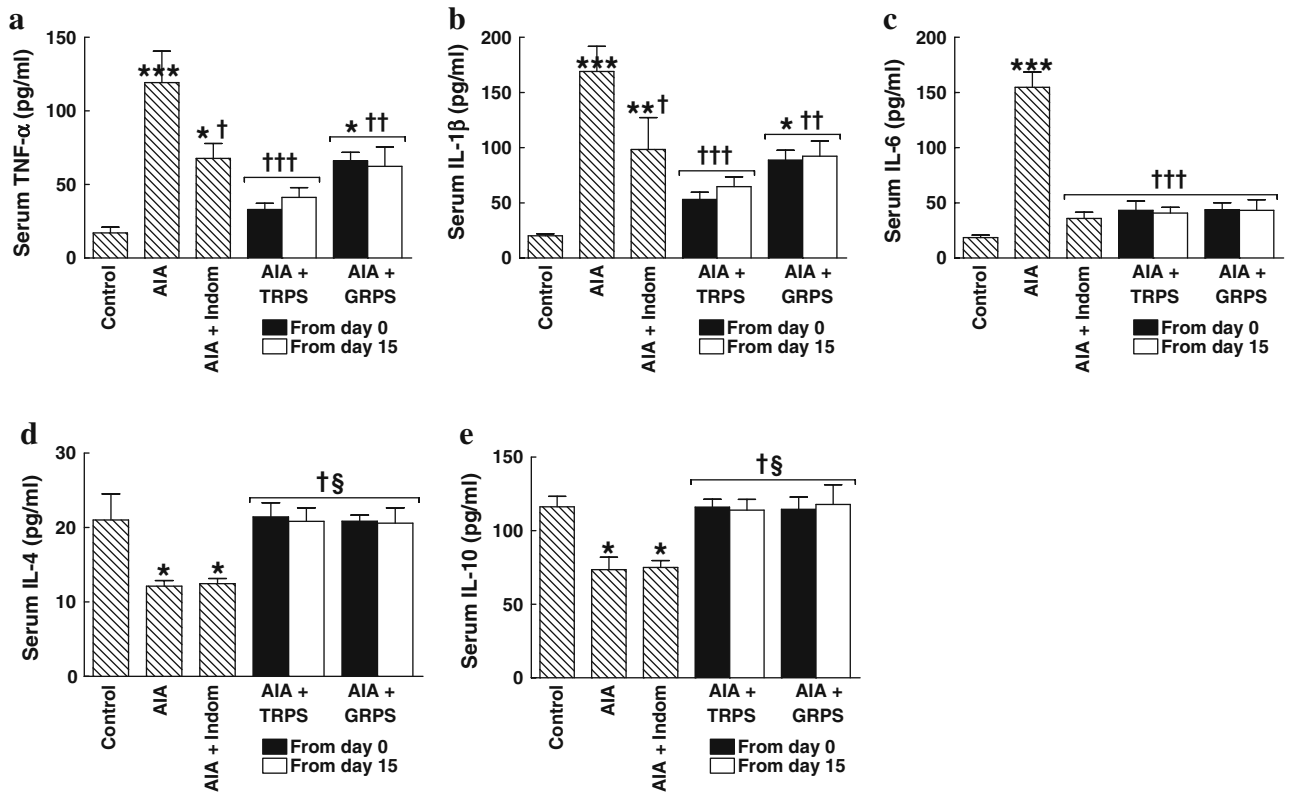


Fig. 4. The modulatory effects of indomethacin, turmeric and ginger on the changes in pro-inflammatory (a–c) and anti-inflammatory (d, e) cytokines of arthritic rats. SEM represented by vertical bars. AIA rat adjuvant-induced arthritis, Indom indomethacin, TRPS turmeric rhizomes powder suspension, GRPS ginger rhizomes powder suspension. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (versus the healthy control group). † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$ (versus the arthritis control group, which received vehicle). § $P < 0.05$ (versus the arthritis group, which received indomethacin).

rats that received TRPS either from days 0 or 15 (Fig. 4). The modulatory effects of TRPS and GRPS on serum anti-inflammatory cytokines were more efficacious than that of indomethacin ($P < 0.05$; Fig. 4d, e).

Modulatory Effects of Turmeric *Versus* Ginger Rhizomes on the Cellular Toxicity and Oxidant Stress in Arthritic Rats

Table 2 revealed that the markers for cellular toxicity (serum ALAT and ALP activities and liver LPO level) significantly increased ($P < 0.05$ to $P < 0.001$), while liver anti-oxidants (CAT and SOD activities and GSH level) significantly decreased ($P < 0.05$ to $P < 0.001$), in arthritic rats that received vehicle compared with the healthy control animals. All of these changes were completely modulated in arthritic rats that received TRPS from day 0 only (Table 2). On the other hand, arthritic rats that received TRPS or GRPS from day 15 or indomethacin did not show any modulation on serum ALAT activity ($P > 0.05$, compared with arthritic control animals). In addition, arthritic rat that received indomethacin, TRPS from day 15 or GRPS from day 0 did not show any modulation on liver GSH level ($P > 0.05$, compared with arthritic control animals). The anti-oxidant activity of TRPS and GRPS were more efficacious ($P < 0.05$ to $P < 0.001$) than that of indomethacin in most cases (Table 2).

The percentages of changes of all parameters measured, compared with the healthy control group, in arthritic groups that received vehicle, indomethacin, TRPS or GRPS from day 0, or TRPS or GRPS from day 15 were 273.2±164.6, 87.4±48.6, 45.0±31.0, 70.1±39.9, 62.9±38.9 and 90.1±53.1, respectively, indicating that the anti-arthritis activity of TRPS exceeded that of GRPS and indomethacin ($P < 0.05$) in AIA rat model.

Beneficial/Deleterious Effects of Turmeric *Versus* Ginger Rhizomes in Healthy Rats

Healthy rats consumed TRPS showed a very highly significant increase in the liver SOD activity ($P < 0.001$) compared with the healthy control animals. On the other hand, healthy rats consumed GRPS showed a significant decrease/increase ($P < 0.05$) in the liver LPO and GHS levels, respectively, compared with the healthy control animals. All other parameters measured in this study did not significantly alter ($P > 0.05$) in healthy rats that received TRPS or GRPS compared with the healthy control animals (data not shown). Moreover, the mortality rates were zero in all groups treated with either TRPS

Table 2. The Modulatory Effects of Indomethacin, Turmeric and Ginger on the Cellular Toxicity and Oxidant Stress in Arthritic Rats

	AIA ⁺						
	Control	AIA	Indom	TRPS		GRPS	
				Day 0	Day 15	Day 0	Day 15
Cellular toxicity markers							
Serum ALAT activity (IU/L)	36.82±1.08	63.77±9.38**	65.14±2.09**	35.55±1.64†§§§	65.78±2.46**	52.90±1.37	65.36±1.79**
Serum ALP activity (IU/L)	11.60±0.65	35.62±11.36*	28.24±2.97	14.12±0.55	15.93±2.28	14.80±1.11	13.93±1.31
Liver LPO (nmol MDA/mg protein)	1.99±0.14	3.43±0.17***	2.11±0.14††	1.81±0.14††	1.78±0.21††	1.17±0.06*††§§	2.53±0.26†
Liver enzymic anti-oxidants							
CAT activity (U/mg protein)	224.7±21.1	112.8±18.4***	224.7±8.6††	160.3±9.3	284.3±23.0††	174.8±20.7	206.9±13.8†
GPx activity (nmol ⁻¹ min ⁻¹ mg ⁻¹ protein)	677.3±105.5	680.5±34.0	717.8±9.2	464.5±39.2	828.0±50.1	1043.0±64.3**††§§	863.3±53.4
GR activity (nmol ⁻¹ min ⁻¹ mg ⁻¹ protein)	33.63±3.43	30.70±1.34	28.80±1.28	31.04±2.87	37.34±1.08§	31.03±0.83	32.74±0.98
SOD activity (mU/mg protein)	4.44±0.35	2.42±0.19*	3.21±0.22	6.88±0.46**††§§§	5.16±0.65††§	6.79±0.47**††§§§	5.95±0.18††§§§
Liver non-enzymic anti-oxidants							
GSH (μmol/mg protein)	70.74±2.83	45.47±2.67***	38.18±3.12***	71.03±1.59††§§§	45.25±3.65***	46.32±2.99***	84.94±4.73††§§§

Values are means±SEM

AIA rat adjuvant-induced arthritis, Indom indomethacin, TRPS turmeric rhizomes powder suspension, GRPS ginger rhizomes powder suspension

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (versus the healthy control group); † $P < 0.05$; †† $P < 0.001$ (versus the arthritis control group, which received vehicle); ‡ $P < 0.05$; ‡‡ $P < 0.01$; ‡‡‡ $P < 0.001$ (versus the arthritis group, which received indomethacin)

or GRPS. Therefore, no deleterious effects were detected for the dose of turmeric and ginger rhizomes used in this study.

DISCUSSION

The present study found that both TRPS and GRPS significantly suppressed the incidence/severity of arthritis by alleviating the hind paws erythema/oedema, bone marrow and spleen hypertrophy, blood lymphocytosis and neutrocytosis, cellular toxicity, and the loss in body weight gain through increasing/decreasing the production of anti-inflammatory/pro-inflammatory cytokines, respectively (Fig. 4), activating the anti-oxidant defence system (Table 2), and increasing the food intake (data not shown). In addition, the present study proves the anti-arthritic activity of turmeric over ginger and indomethacin, which has not been established before. The percentage of disease recovery was 4.6–8.3% and 10.2% more in arthritic rats that received TRPS compared with those that received ginger or indomethacin ($P < 0.05$), respectively.

TNF- α is a pleiotropic cytokine, which produces primarily by monocytes/macrophages and plays a critical role in both acute and chronic inflammation. It facilitates inflammatory cell infiltration by promoting adhesion of neutrophils and lymphocytes to endothelial cells [5]. In addition, it induces the production of IL-1 β , IL-6 and macrophage chemotactic protein-1 (MCP-1). TNF- α is found in the sera and synovial fluid of RA patients and its level has been associated with clinical and laboratory markers of RA disease severity. Also, it involved in the early perpetuation and in the maintenance of synovitis in AIA [5]. When TNF- α is specifically blocked, the severity of inflammation is reduced [4]. IL-1 β is mainly secreted by macrophages and many of its inflammatory effects overlap with those of TNF- α described above [5]. Also, TNF- α and IL-1 β induce receptor activator of nuclear factor- κ B ligand on macrophages and stimulate their differentiation into osteoclasts that resorb and destroy bone [6]. IL-6 produced mainly by monocytes, T-cells and fibroblasts. It stimulates MCP-1 secretion and induces osteoclast differentiation [5, 33]. TNF- α , IL-1 β and IL-6 are involved in the development of clinical symptoms in CFA treated rats, including body weight loss, joint swelling and blood neutrocytosis [5]. IL-4 and IL-10 (Th2 anti-inflammatory cytokines) have been thought to be upstream regulators that control the progression of RA negatively. IL-4 enhances the syn-

thesis and the production of tissue inhibitors of MMPs in human mononuclear phagocytes and cartilage explants, and hence suppresses MMPs activation, while IL-10 inhibits the cytokine production and release by activated macrophages [34, 35]. In the present study, all changes in pro-inflammatory and anti-inflammatory cytokines of arthritic rats were completely prevented in arthritic rats that received TRPS only (Fig. 4).

The dried rhizomes of turmeric (rich in phenolic curcuminoids: curcumin, demethoxycurcumin and bis-demethoxycurcumin) and ginger (rich in pungent phenolic compounds: gingerols and shogaols) or extracts thereof are important ingredients of many traditional/alternative medicines worldwide [19, 20]. Modern science has revealed that turmeric curcuminoids inhibit inflammation by blocking the adhesion of monocytes to endothelial cells through inhibiting the activation of the cell surface adhesion molecules. Also, they suppress inflammation by inhibiting the nuclear factor- κ B activation, which leads to inhibition of gene expression of pro-inflammatory cytokines (TNF- α and IL-1 β), chemokines (such as MCP-1) and cyclooxygenase (COX)-2 as well as osteoclastogenesis [15, 19, 36]. COX-2, which converts arachidonic acid into prostaglandins (PG) such as PGE₂, is abundantly expressed in the synovial explants of RA by TNF- α and IL-1 β [37]. PG greatly potentiate exudates by inducing relaxation of arteriolar smooth muscles and increasing the blood supply to tissues [9]. Also, both COX-2 and PGE₂ induce activation and growth of synovial fibroblasts, which associated with hyperplasia and pannus formation, by inhibiting apoptosis. Therefore, inhibition of COX-2 and PGE₂ by curcuminoids has a marked impact on the prevention/alleviation of RA progression [37]. Gingerols and shogaols inhibit the induction of several genes involved in inflammatory response; some of these genes encode pro-inflammatory cytokines, chemokines and the inducible enzyme COX-2 [15–17]. Gingerols can also inhibit the synthesis of inflammatory mediators such as PG and leukotrienes *in vitro* [16]. In addition, ginger extract was found to inhibit beta-amyloid peptide-induced cytokine and chemokine expression in cell line of human monocytes [38]. The expression of MCP-1 and IFN- γ -activated protein (IP-10) in human synoviocytes was suppressed by ginger extract [18]. MPC-1 is a potent chemotactic agent for monocytes/macrophages and has also been implicated in inducing MMPs [39, 40]. IP-10 is a chemoattractant for activated Th1 cells and selectively promotes dominance of IFN- γ /Th1 over IL-4/Th2 responses [41]. Therefore, inhibition of MPC-1 and IP-

10 expression in synoviocytes by ginger extract have a marked impact on the prevention and alleviation of RA progression [18]. Our results are consistent with the above reports which may explain the anti-inflammatory effects of turmeric and ginger rhizomes shown in this study. The subtle increase in the anti-inflammatory activity of TRPS *versus* GRPS shown here in AIA rat model may be due to that turmeric contains more components that act synergistically to alleviate inflammation or its components (*e.g.* curcuminoids) have stronger anti-inflammatory activity compared with that in ginger (*e.g.* gingerols and shogaols). Also, it may be due to the subtle higher percentage of curcuminoids (3–5%) in turmeric rhizomes compared with their natural analogue gingerols and shogaols (1–3%) found in ginger rhizomes.

ROS are able to destroy membrane lipids, proteins, DNA and cartilage [9]. Aerobic cells were endowed with extensive anti-oxidant defence mechanisms to counteract the damaging of ROS. SOD is the most important anti-oxidant enzyme since it catalyses the dismutation of O_2^- to H_2O_2 , which is degraded by either catalase or selenoenzyme GPx. GSH, the first line defence against lipid peroxidation, is an essential electron donor to GPx in the reduction of hydroperoxides and serves as a nucleophilic co-substrate to glutathione transferases in the detoxification of xenobiotics. GR is necessary for the GSH redox cycle, which maintains adequate levels of cellular GSH [42]. The uncontrolled production of ROS (especially superoxide anion and hydroxyl radicals) by phagocytic cells due to the inflammatory surge in RA leads to a decrease in SOD and CAT activities in addition to GSH level as a consequence of their supersaturation and consumption during oxidative stress as well as loss through cellular lysis, which resulted from membrane lipid peroxidation and lysosomal destruction [43, 44]. The significant increase in serum ALAT and ALP activities as well as liver LPO level (sensitive markers of cellular integrity and toxicity) of arthritic rats in the present study are in agreement with the reported significant decrease in lysosomal stability of CFA treated rats [21]. Elevated level of serum ALP activity in arthritic rats can also be due to the increase in bone erosion and periarticular osteopenia, since the enzyme is released into circulation in the course of bone resorption and formation [45]. The significant decrease/increase in the cellular toxicity markers (LPO, ALAT and ALP) and anti-oxidant defence system (SOD, CAT and GSH), respectively, shown in arthritic rats that received TRPS or GRPS especially from the day of arthritis induction emphasises

the role of these plants in preventing organs damage and bone loss in AIA rat model through scavenging the free radicals. Another study reported that curcumin and its analogues were effective in scavenging free radicals, which induce oxidative haemolysis of human erythrocytes [46]. Also, it was found that addition of ginger (1%) to normal diet prevented the formation of free radicals and maintained the integrity of erythrocytes in rats [47].

The anti-inflammatory and anti-oxidant effects of TRPS and GRPS shown in this study were more efficacious than that of indomethacin ($P < 0.05$ to $P < 0.001$), especially when the treatment started from day 0 than day 15. NSAIDs (such as indomethacin) reduce inflammation, swelling and arthritic pain by inhibiting PG synthesis and/or production through selective inhibition of COX-1 and COX-2 over lipoxygenases. Inhibition of COX-1 induces adverse effects such as gastrointestinal/renal injury [48]. On the other hand, turmeric and ginger components (such as curcumin and shogaols, respectively) have pharmacological properties mimicking dual-acting NSAIDs (dual inhibition of the pro-inflammatory enzymes selectively: COX-2 and 5-lipoxygenase) in intact human leucocytes [15, 16]. Such dual-acting NSAIDs are more effective than conventional NSAIDs and have fewer or no side effects [49]. This explains the higher/lower anti-arthritis/harmful effects, respectively, shown for both TRPS and GRPS in this study compared with indomethacin. In general, turmeric and ginger are considered to be safe herbal medicine with only few and insignificant adverse/side effects. The dose used in the present study is equivalent to 2 g/day/human and did not induce any deleterious effects in healthy rats. Dietary intake of these spices is estimated to be 2–4 g/day, while up to 8 g/day of curcumin (that constitutes ~3% of turmeric rhizomes) and 6 g/day of ginger are without evidence of side effects in human [15, 16].

In conclusion, turmeric rhizome was more effective in alleviating the inflammatory immune response and oxidant stress in rat model of human RA than ginger rhizome and indomethacin. Therefore, turmeric rhizomes dietary supplements may have beneficial effects against arthritis progression in RA patients.

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Author contributions. G. R. planned the study (with assistance from M. A. A.-K.), designed and carried out all experiments (except for that in Table 2, which were carried out by W. M. E.-S.), performed the statistical analysis and summarized the results. G. R. drafted the manuscript with assistance from M. A. A.-K. and W. M. E.-S.

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