ORYZA OIL & FAT CHEMICAL CO., LTD.

RED GINGER EXTRACT

An All Natural Anti-arthritic & Anti-inflammatory Agent for Food & Cosmetics Applications

- **RED GINGER EXTRACT-P**
  (Powder, Food Grade)
- **RED GINGER EXTRACT-WSP**
  (Water-soluble Powder, Food Grade)
- **RED GINGER EXTRACT-PC**
  (Powder, Cosmetic Grade)
- **RED GINGER EXTRACT-WSPC**
  (Water-soluble Powder, Cosmetic Grade)
- **RED GINGER EXTRACT-LC**
  (Water-soluble Liquid, Cosmetic Grade)

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ver. 2.1 HS

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1. Introduction

Rheumatism and knee osteoarthritis (usually referred to as "arthropathy") are common degenerative disorders of aging. The early stage of rheumatism is characterized by the inflammation of genetic and immunologic origins of the synovial membrane which lines the joint capsules. As inflammation progresses, synovial fluid pools, pannus (increased synovial membrane) is formed followed by destruction of cartilage. At the advance stage, joints are filled with synovial villus tissue without proper functioning. Upon progression of symptoms, patients will experience severe pain, joints deformities coupled with mental stress. The conventional treatment prescribed for rheumatism includes: Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), steroids, SH compounds and immunosuppresive agents. The commonly prescribed latest NSAIDS group - Cyclooxygenase-2 (COX-2) selective agents usually require 1-3 months to be effective while posting side effects on hepatic functions & hypoactive immune system. On the other hand, osteoarthritis is characterized by the deterioration of cartilage in the joints (i.e., intersections of two bones), resulting in pain and loss of function. The condition primarily affects weight-bearing joints such as the knees, hips, feet, and back, and the joints in the fingers and hands. As the disease progresses, crevices and bone spurs (**osteophytes**) may develop within the affected joint, increasing pain and decreasing mobility. NSAIDS are commonly prescribed for pain relief while nutritional supplement (e.g. chondroitin, glucosamine) may be used as part of an overall treatment program to reduce symptoms of osteoarthritis.

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Pathophysiology of Arthritis

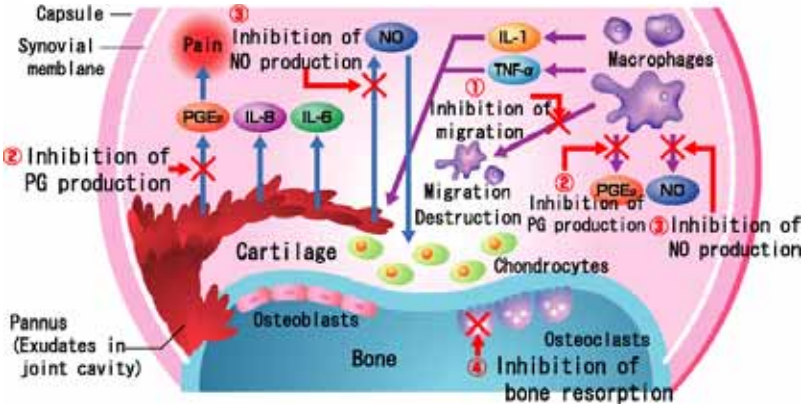

Fig. 1. Pathophysiology of Arthritis

It is estimated that there are more than 750,000 arthropathic people in Japan and 6.6 million in the US. Nutritional supplements (e.g. glucosamine) are commonly used for the prevention and pain relief. However, there is insufficient research data to prove the benefits of this treatment. In addition, animal derived Type II collagen has been used in the treatment of rheumatism as Cartilage types II collagen was strongly expressed in late-stage specimens, reflecting the high matrix-remodelling activity of advanced osteoarthritic cartilage. Other commonly used supplements include chondroitin sulphate, hyaluronic acid, methylsulfonyl methane (MSM), cat's claw (TNF-α & PGE_2 supressor) and white willow.

Ginger (*Zingiber Officinale* Roscoe) is commonly found in OTC preparations as an aromatic stomachic, anti-nausea and pain relief remedies. Actives found in ginger essential oil include gingerols and shogaols [1)] which are vanilloids [2)] that bind to capsaicin-sensitive parasympathetic nerve [3)]. The pharmacological properties of these compounds has been identified to be anti-inflammatory that suppress platelet aggregation [4)] and nitric oxide production [5)] while inhibit COX-2 activity [6)].

Red Ginger (*Z. officinale* var. Rubra) is a variance of the *Zingiber Officinale* species cultivated in Indonesia and Malaysia. Besides having gingerols and shogaols, it is loaded with anthocyanin and tannin in its root bark. Red Ginger is reddish-violet in colour and known as "Jahe Merah" by the natives (Fig. 2). Traditionally, it is used in spices, syrup and as remedy for rheumatism, osteoporosis, asthma & cough. Recent reports on the anti-inflammatory effect [7-9)] of anthocyanin of *Z. Officinale* received much attention. Oryza Oil & Fat Chemical Co., Ltd. prompted researches into evaluating the anti-inflammatory effect of Red Ginger with various experimental models and results revealed that aqueous ethanol extract of Red Ginger is beneficial against acute and chronic inflammations. In addition, human study revealed that Red Ginger Extract lowered serum hyaluronic acid, hence prevents destruction of joint cellular matrix component.

Red Ginger Extract is an all natural analgesic & anti-inflammatory agent suitable for the application of anti-arthritic preparations.

Fig. 2. Red Ginger (*Z. Officinale* var. Rubra)

References）

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2) Dedov V. N., Tran V. H., Duke C. C., Connor M., Christie M. J., Mandadi S., Roufogalis B.D. Gingerols: a novel class of vanilloid receptor (VR1) agonists..*Br. J. Pharmacol.* **137**, 793-798 (2002).
3) Someya A., Horie S., Yamamoto H., Murayama T. Modifications of capsaicin-sensitive neurons in isolated guinea pig ileum by [6]-gingerol and lafutidine. *J. Pharmacol. Sci.* **92**, 359-366 (2003).
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5) Ippoushi K., Azuma K., Ito H., Horie H., Higashio H. [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life Sci.* **73**, 3427-3437 (2003).
6) Kim S. O., Kundu J. K., Shin Y. K., Park J. H., Cho M. H., Kim T. Y., Surh Y. [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-κB in phorbol ester-stimulated mouse skin *J. Oncogene.* **24**, 2558-2567 (2005).
7) Rossi A., Serraino I., Dugo P., Di Paola R., Mondello L., Genovese T., Morabito D., Dugo G., Sautebin L., Caputi A. P., Cuzzocrea S. Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radic. Res*. **37**, 891-900 (2003).
8) Tsuda T., Horio F., Osawa T. Cyanidin 3-*O*-β-D-glucoside suppresses nitric oxide production during a zymosan treatment in rats. *J. Nutr. Sci. Vitaminol.* (*Tokyo*) **48**, 305-310 (2002).
9) Li W. G., Zhang X. Y., Wu Y. J., Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds.*Tian X. Acta. Pharmacol. Sin.* **22**, 1117-1120 (2001).

2. Physiological Compounds of RED GINGER EXTRACT

Common to the *Z. Officinale* family, Red Ginger Extract is rich in gingerols & shogaols as illustrated in Fig. 3. Meanwhile, **[6]-gingerol** and **3*R*,5*S*-[6]-gingerdiol** are characteristic compounds of Red ginger Extract due to its high concentration as compared to other *Z. Officinale* species.

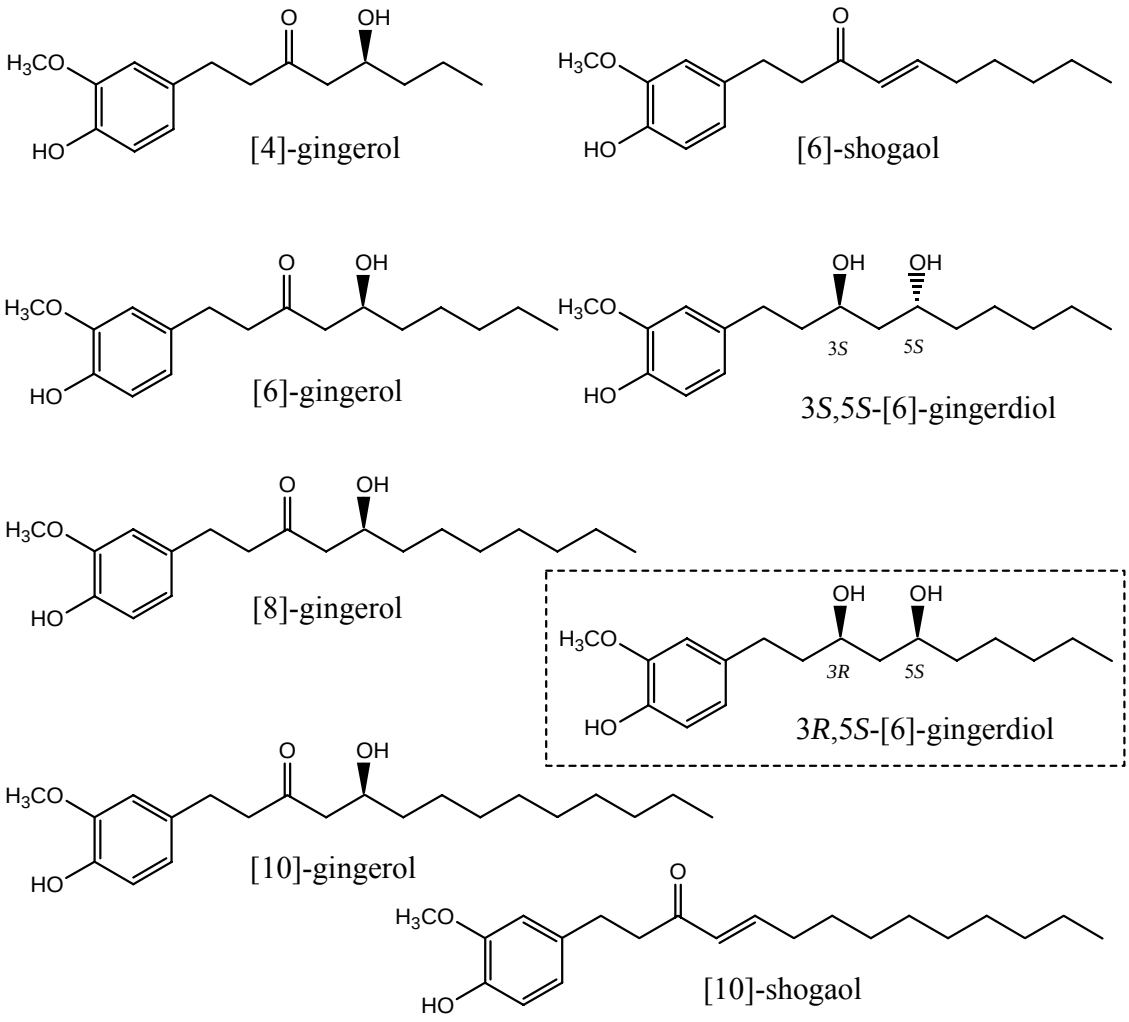

Fig. 3. Physiological Compounds of Red Ginger Extract

3. Anti-arthritic effect of RED GINGER EXTRACT

(1) Effect on acute and chronic inflammation

1) Acetic Acid Induced Abdominal Inflammation in Mice

The effect of Red Ginger Extract (non binder, [6]-gingerol 2%, tannin 2%; this preparation is used in the following experiments) on acute inflammation was experimented on acetic acid induced abdominal inflammation model in mice. The analgesic effect of Red Ginger Extract was determined by Writhing Counts.

As illustrated in Fig. 4, oral administration of Red Ginger Extract (10 – 100mg/kg) demonstrated a dose dependent pain relief effect with significant reduction in Writhing counts. Similarly, it exerted a dose-dependent anti-inflammatory effect where the amount of leaked dye, an indicator on extend of inflammation, reduced significantly at 50 and 100mg/kg. In addition, Young H-Y *et al.,* showed that intraperitoneal injection of [6]-gingerol (50mg/kg) relieved pain by 50% [†)]. As revealed in this experiment, Red Ginger Extract in low oral dose of 0.2 – 2mg/kg demonstrated effective analgesic and anti-inflammatory effects suggesting the synergisms of various physiological compounds presents in Red Ginger Extract.

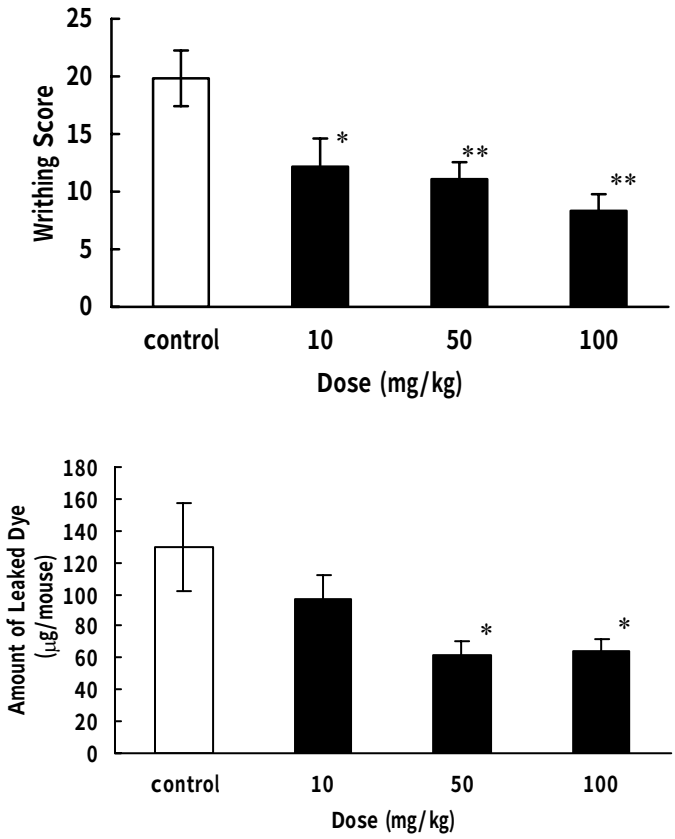

Fig. 4. The effect of Red Ginger Extract on acetic acid induced abdominal inflammation in mice.

(Upper graph: The Analgesic Effect of Red Ginger Extract on Writhing counts
Lower graph: The Anti-inflammatory Effect of Red Ginger Extract)

Each column represents the mean value with S.E. (n=12).

"*" denotes significant difference from control where *: $p<0.05$, **: $p<0.01$

[Method]
Red Ginger Extract was given orally to fasting mice (ddy, male, 5-wk old) followed by I.V. injection of Pontamin Sky Blue 2%(10mL/kg), an indicator of inflammation 55min later. Inflammation was induced 5min later by acetic acid 1% (10mL/kg) via intraperitoneal injection. Frequency of Writhing events was counted for 15min followed by dissection of abdominal cavity under ether anesthesia. Abdominal cavity containing dye of Pontamin Sky Blue was flushed with saline (8mL). Leaked dye was measured and calibrated to 10mL for absorbance measurement at wavelength 590nm.
†: Young H-Y., Luo Y-L., Cheng H-Y., HsiehW-C, Liao J-C., Peng W-H. Analgestic and anti-inflammatory activities of [6]-gingerol. *J. Ethnopharmacol.* **96**, 207-210 (2005).

2) Rat Adjuvant Arthritic Model
The effect of Red Ginger Extract on chronic inflammation was experimented using rat adjuvant arthritis model. This model is generally used for the diagnosis of rheumatism. As shown in Fig. 5, edema was induced and observed in rats treated with adjuvant (control). No significant changes observed in group treated with Red Ginger Extract 1mg/kg. However, marked anti-inflammatory effect was observed on day-13 in group treated with Red Ginger Extract 10mg/kg. Indomethacin (0.5mg/kg), a commonly prescribed NSAIDS drug was used as positive control, showing a significant anti-inflammatory effect ($p<0.01$) upon induction of inflammation throughout end of experiment.

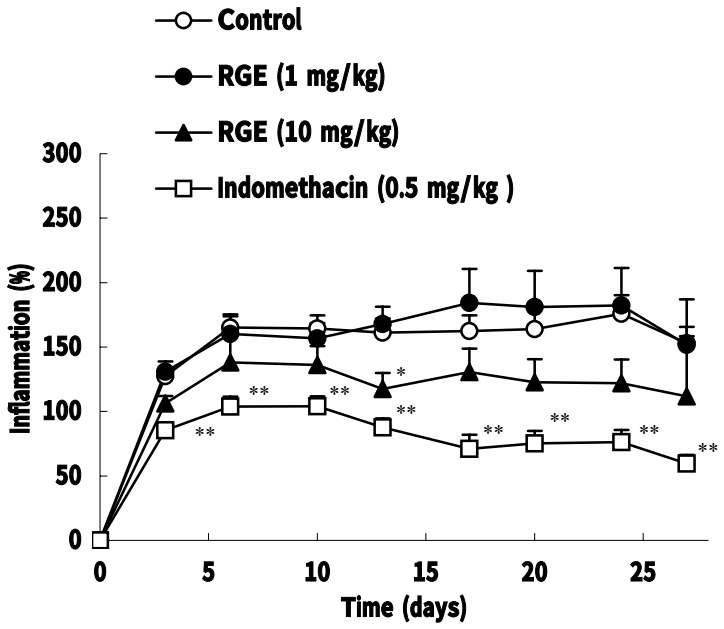

Fig. 5. The Effect of Red Ginger Extract (RGE) on Rat Adjuvant Arthritis Model.
Each point represents the mean value with S.E. of 7 rats. "*" denotes differences from control, *: $p<0.05$; **: $p<0.01$ respectively.

X-ray images of limbs of rat treated with adjuvant were taken. Fig. 6 showed that severe joint destruction observed in control group with adjuvant while joint destruction in group treated with Red Ginger Extract & Indomethacin was less severe.

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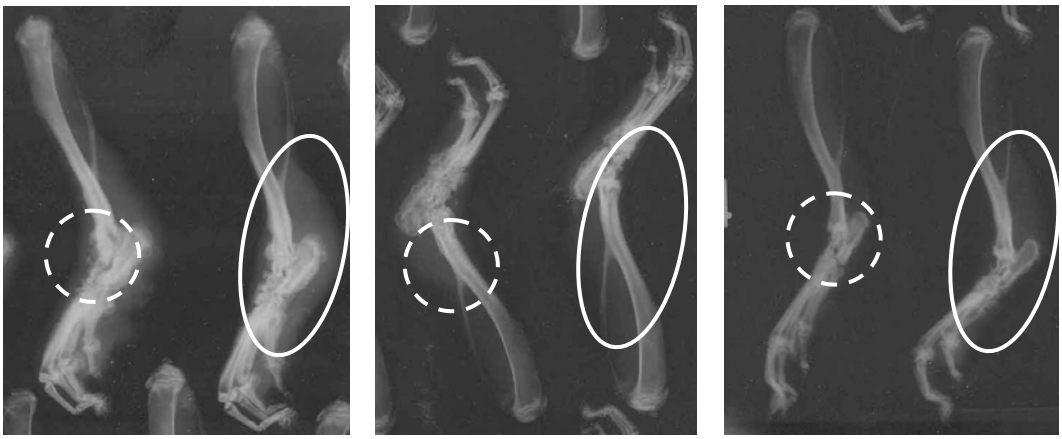

Control RED GINGER EXTRACT 10 mg/kg Indomethacin 0.5 mg/kg

Fig. 6. X-ray images of limbs of rat treated with adjuvant.
Solid line: Edema Dashed line: destruction of joint

Joint specimens were examined under microscope and revealed evidence of joint destructions accrues where synovium thickens, the cartilage and the underlying bone begins to disintegrate (as in the control group). Meanwhile, less destruction observed in bone tissues of samples treated with Red Ginger Extract 10mg/kg (Fig. 7). Red Ginger Extract prevents autoimmune inflammatory disorders.

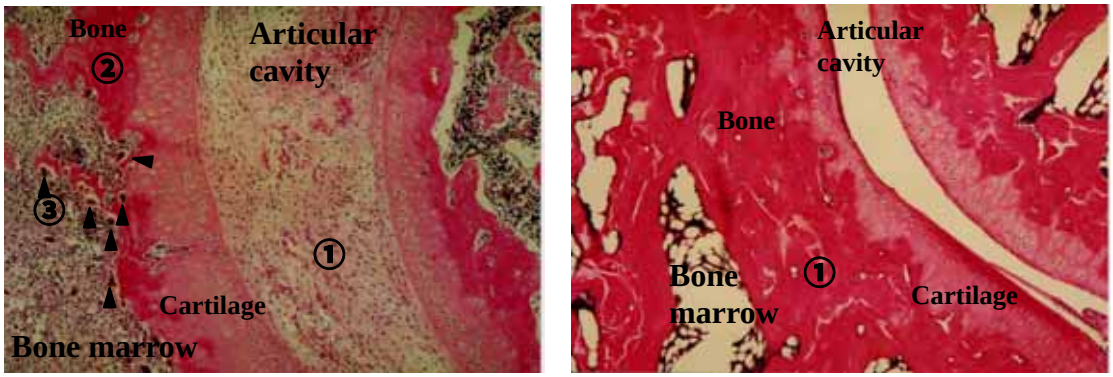

Fig. 7. Microscopic illustration of joint tissues of rats treated with adjuvant (H.E. stain, x10).
Left: Control [① papillary growth of villus, ② Medium-level of bone destruction, ③ increased of osteoclast cells]
Right: Sample treated with Red Ginger Extract (10mg/kg) [① bone tissue was normal, less destruction]

[Method]

S.C injection of 0.1ml Freund's complete adjuvant (Difco, 1ml) containing inactivated strains of *Mycobacterium butyricum* (Difco, 10mg) was introduced to the rear of right limbs of rats (SD, male, 8 week old). Samples of Red Ginger Extract were given daily after immunization and edema was determined by measuring volume of limbs treated with adjuvant.

3) Arthritis Model of Joint Cellular Matrix in Mice

Further research was prompted to evaluate the effect of Red Ginger Extract on cellular matrix of arthritic joint in mice. Type II collagen was introduced as antigen in this model. Fig. 8 illustrated that inflammation reduced significantly ($p<0.05$) with increasing concentration of Red Ginger Extract.

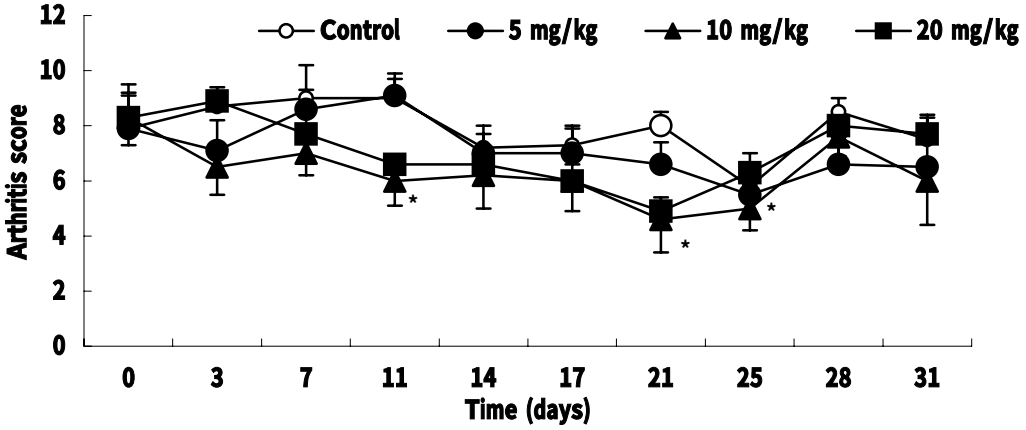

Fig. 8. The Effect of Red Ginger Extract on Cellular Matrix of Arthritic Joints in Mice

Each point represents the mean value with S.E. of 7 mice. "*" denotes significance difference from control, *: $p<0.05$

[Method]

Equal volume of Bovine Type II collagen and Freund's Complete Adjuvant (100μL) was intradermally injected to the lower limbs of mice (male DBA/1J, 5-wk old). Above booster immunization was prepared similarly and re-introduced 3 weeks later. Mice were divided into 4 groups according to the levels of inflammation. Samples of Red Ginger Extract were given orally at different concentration (5, 10 & 20mg/kg) respectively on a daily basis. Meanwhile, 5% acacia gum suspension was used as control. Intensity of inflammation was determined every 3-4 days during 31-day of samples administration. Intensity of inflammation was determined according to Banerjee S *et al.*[†)] as arthritis score in five levels based on average of total scores of 4 legs (16 at maximum).

†) Banerjee S., Haqqi T. M., Luthra H. S., Stuart J. M., David C. S. Possible role of V□ T cell receptor genes in susceptibility to collagen-induced arthritis in mice. *J. Exp. Med*. **167**, 832-839 (1988).

(2) Anti-inflammatory effect of Red Ginger Extract – Mechanism of Actions

Inflammation is triggered in response to mechanical, chemical or immunological challenges. Cascade of inflammatory cells are released upon infiltration of macrophages into injured tissue (e.g. pro-inflammatory cytokines, prostaglandins [PG]) resulting in pain, tenderness and swelling in the affected injured tissue. Further in-depth research was prompted to evaluate the effect of Red Ginger Extract on migration of macrophages, PG production and cyclo-oxygenase activity.

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1) Inhibition of Macrophage Migration

The effect of Red Ginger Extract on the release of monocytes (which will differentiate into macrophage) was experimented with TAXIScan (Effector Cell Institute). As illustrated in Fig. 9 & 10, Red Ginger Extract demonstrated a does-dependent suppression on the migration of monocytes of human peripheral blood which was stimulated by monocyte chemotactic factor (MCP-1, 10nm).

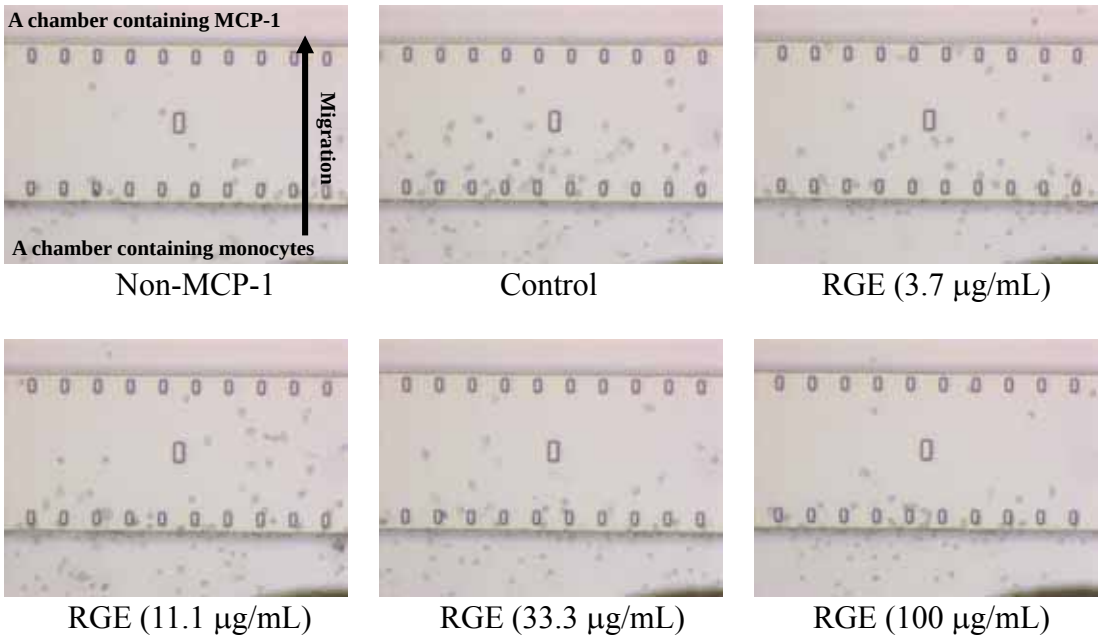


Fig. 9. Migration of monocytes in chambers of TAXIScan
(amorphous particles are cells)

[Method]

Crude fraction of monocytes was obtained from treatment of dextran & Lymphorep on human peripheral blood. Crude fraction of monocytes was suspended in a medium where CD13 & CD19 micro beads were added for placement in LD column (Myltenyi Biotec) for collection of the non-absorbing fraction – fraction of monocyte. Density of monocytes was adjusted to $2x10^6$ pieces/ml followed by the addition of Red Ginger Extract and incubation for 1 hour at 37°C. Migration of monocytes was triggered with MCP-1 (10nm) and results was observed in TAXIScan (Effector Cell Institute).

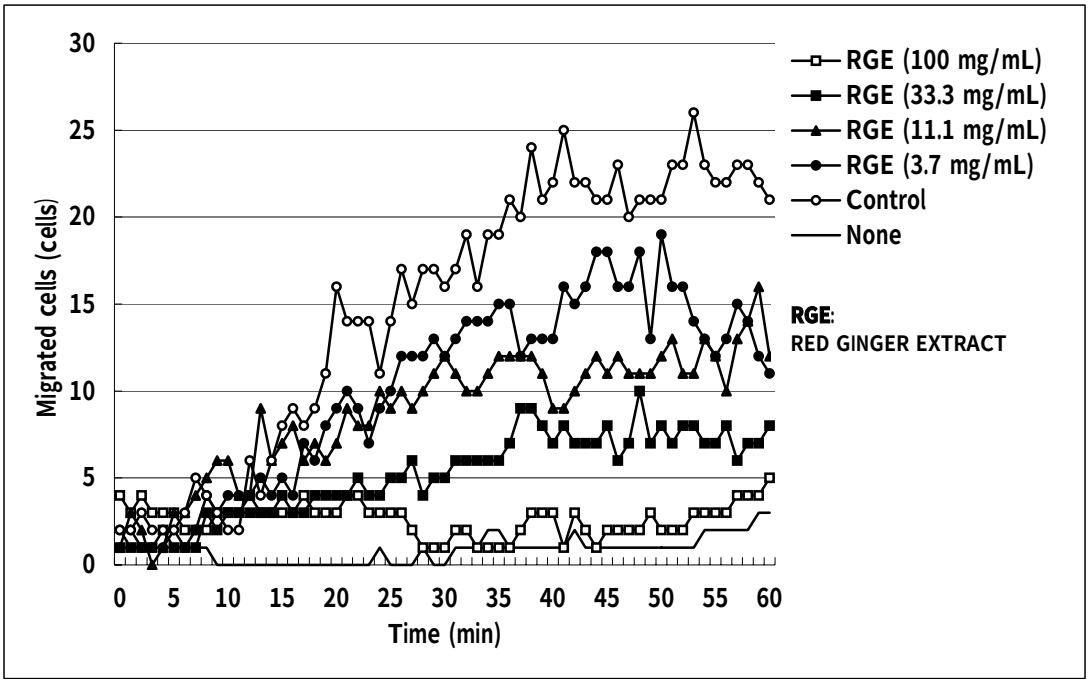


Fig. 10, The Effect of Red Ginger Extract on the migration of human monocytes triggered with MCP-1.

2) Inhibition of prostaglandins E_2(PGE_2) production

Further experiment was prompted to study the effect of Red Ginger Extract on PG production. Cultured macrophage cells (RAW264.7) were stimulated by lipopolysaccharide (LPS) to produce prostaglandins (PG). Results showed that Red Ginger Extract significantly suppressed the production of PGE_2 from cells RAW264.7 at concentration of 3 & 10μg/ml. Meanwhile, no cytotoxicity occurred at these concentrations.

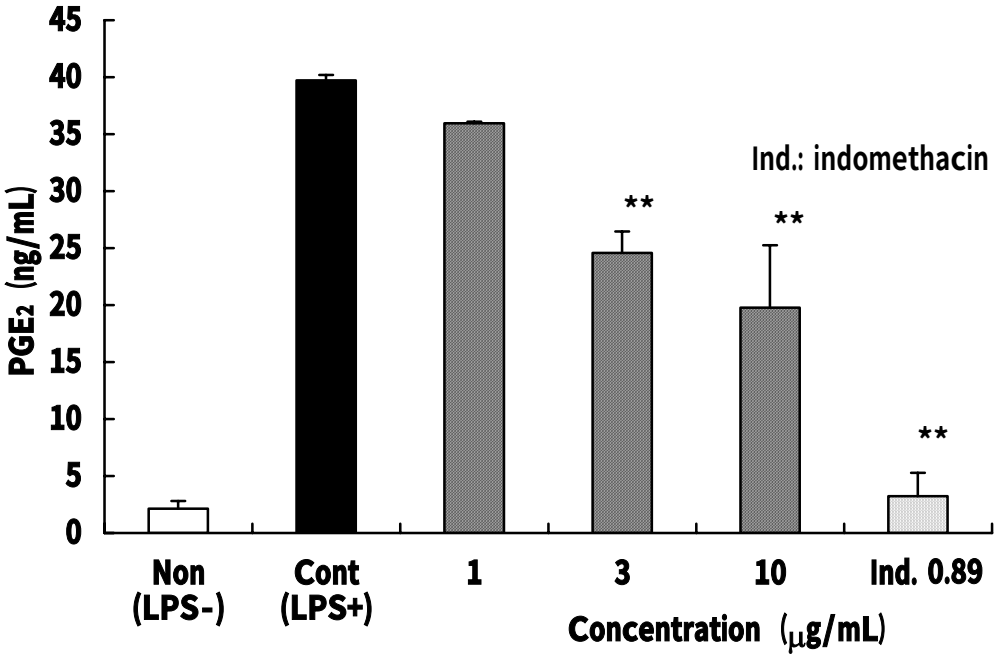


Fig. 11, The effect of Red Ginger Extract on PGE_2 production
Each column represents the mean value with S.E. of 3 experiments.
"*" denotes significant difference from control. **: $p<0.01$

[Method]

RAW264.7 cells was suspended in MEM medium containing 0.1mM non-essential amino acids, 10% fetal bovine serum, penicillin (100units/mL) and streptomycin (100μg/mL) at concentration of $1x10^6$ cells/mL. 200μl of the suspension was cultured in a 48-welled plate for 24 hours followed by rinsing in serum-free medium (200μl). Later, serum-free medium (170μl) was added to each well followed by the addition of 10μl LPS (200μg/ml; *E.coli* serotype 0127:B8, Sigma) solution and continue culture for 20 hours. Supernatant layer was collected upon completion of culture for the measurement of PGE_2 with Prostaglandin E_2 EIA Kit Monoclonal (Cayman Chemical Co.)

3) Selective Inhibition of COX-2

In-depth research was fostered to understand the anti-inflammatory effect of Red Ginger Extract by studying its effect on COX-1 & COX-2 *in-vitro*. As illustrated below, Red Ginger Extract exerted a dose-dependent (1 to 10μg/ml) effect on PG production with COX-1. In contrary, Red Ginger Extract suppressed PG production *in-vitro* at concentration of 3 and 10μg/ml.

The discovery on the stimulatory effect of Red Ginger Extract on COX-1 is indeed interesting. Kim *et al.* [1)] revealed that [6]-gingerol enhances COX-2 expression in mouse skin stimulated by phorbol ester while exert no effect on COX-1. Alternatively, research leaded by Nurtjahja-Tjendraputra [2)] revealed that [6]-gingerol or its related compounds inhibited platelet aggregation via its suppression on COX-1 activity. This indicates that besides gingerol, other physiological components of Red Ginger Extract enhanced the effect of COX-1 leading to the production of PG which is protective to the gastric membrane. Hence, it is believed that Red Ginger Extract exerts selective anti-inflammatory effect on COX-2 inhibition similar to that of NSAIDS while protective on gastric membrane.

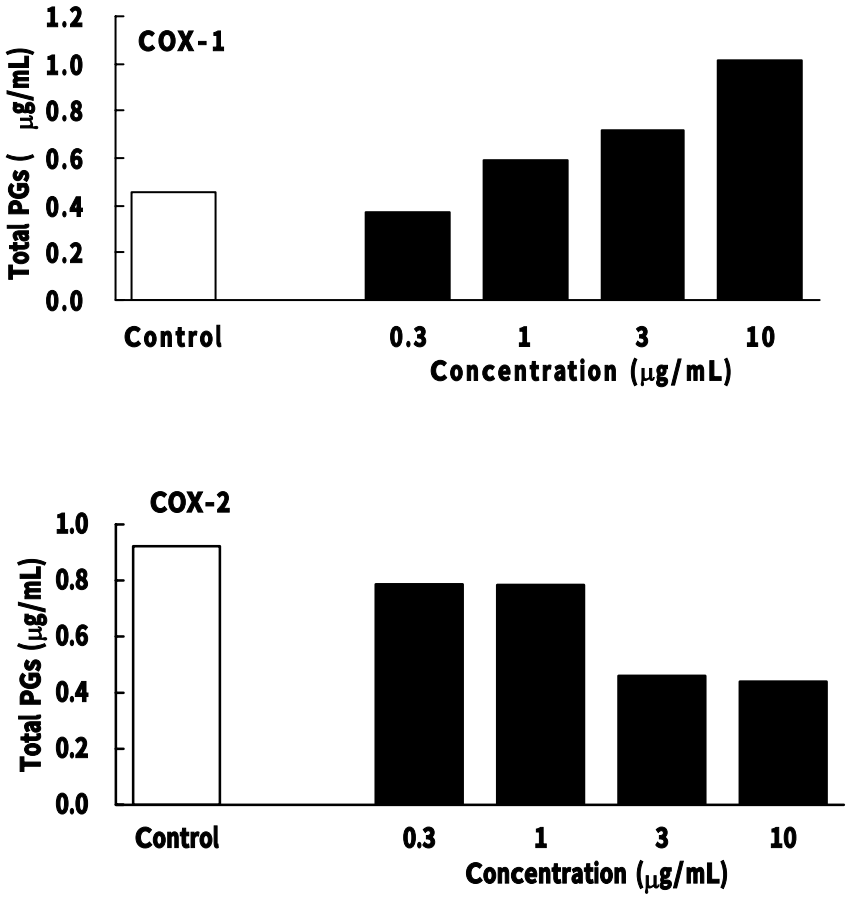


Fig. 12. The effect of Red Ginger Extract on COX-1 & COX-2.
Each column represents the mean value of 2 experiments.

1) Kim S. O., Kundu J. K., Shin Y. K., Park J. H., Cho M. H., Kim T. Y., Surh Y. [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-κB in phorbol ester-stimulated mouse skin *J. Oncogene.* **24**, 2558-2567 (2005).
2) 2) Nurtjahja-Tjendraputra E., Ammit A. J., Roufogalis B. D., Tran V. H. Duke C. C. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thromb. Res*., **111**, 259-265 (2003).

[Method]
Commercially available COX Inhibitor Screening Assay Kit (Cayman Chemical Co.) was used for the above experiments.

4) The effect of Red Ginger Extract on the inhibition of NO production
NO (nitric oxide) is produced by macrophage or damage cartilage cells upon injury. Experiments are prompted to study the effect of Red Ginger Extract on NO production from RAW264.7 cells triggered with LPS. Results showed that 100μg/ml of Red Ginger Extract suppressed the production of nitrogen monoxide by approximately 50%. It was found that [6]-shogaol and [6]-gingerdiols exerted potent inhibitory effect on NO production instead of its principal component, gingerols. Besides, tannin fraction (tannin 5.8%) isolated from Red Ginger Extract demonstrated similar inhibitory effect against NO production. Hence, the essential oil components and tannin of Red Ginger Extract are inhibitory against NO production by macrophage..

Table 1. The effect of Red Ginger Extract on the inhibition of NO production

.	Upper：NO in supernatant（μM）, mean±SD Lower：Inhibition（%）						
Conc.（μg/mL）	0（LPS-）	0（LPS+）	1	3	10	30	100
RED GINGER EXTRACT	1.04±0.20	4.11±0.40	3.87±0.34 (7.8)	3.84±0.23 (8.8)	3.81±0.30 (9.8)	3.59±0.26 (16.9)	2.59±0.29 (49.5)
[4]-gingerol	1.17±0.18	4.77±0.28	4.98±0.22	5.10±0.24	4.98±0.22	5.03±0.45	4.98±0.26
[6]-gingerol	0.96±0.14	3.21±0.27	3.50±0.26	3.66±0.33	3.27±0.26	3.11±0.22 (4.3)	2.81±0.47 (17.6)
[8]-gingerol	0.72±0.18	4.34±0.21	4.17±0.27 (4.7)	4.06±0.24 (7.7)	3.86±0.32 (13.3)	3.59±0.21 (20.7)	3.20±0.17 (31.5)
[10]-gingerol	1.12±0.43	4.20±0.17	4.44±0.27	4.32±0.16	4.33±0.50	4.28±0.72	3.55±0.08 (21.1)
[6]-shogaol	0.98±0.17	3.56±0.30	3.81±0.29	3.60±0.29	3.21±0.31 (13.6)	2.79±0.09 (29.8)	1.22±0.04 **(90.7)**
3*S*,5*S*-[6]-gingerdiol	0.76±0.12	3.57±0.29	3.53±0.19 (1.4)	3.50±0.24 (2.5)	3.35±0.14 (7.8)	2.98±0.11 (21.0)	1.36±0.13 **(78.7)**
3*R*,5*S*-[6]-gingerdiol	0.88±0.11	3.71±0.22	3.65±0.41 (2.1)	3.64±0.39 (2.5)	3.56±0.35 (5.3)	3.43±0.32 (9.9)	1.60±0.11 **(74.6)**
[10]-shogaol	1.21±0.18	3.26±0.64	2.78±0.10 (23.4)	2.88±0.11 (18.5)	2.76±0.10 (24.4)	2.68±0.70 (28.3)	2.62±0.71 (31.2)
Tannin fraction（5.8% tannin）	1.12±0.29	4.18±0.31	4.29±0.19	4.13±0.37 (1.7)	3.97±0.32 (6.9)	3.85±0.39 (10.9)	3.10±0.37 **(35.4)**

N＝6

[Method]
200µl of pre-cultured RAW264.7 cells was incubated in 48-welled plate for 24-hour followed by change of medium to serum free medium. LPS (final concentration 20µM) and sample solution of Red Ginger Extract was added and continue cultured for another 24-hour. Last, 100µl of supernatant layer was collected for determination of NO contents using Grease reagent.

Based on the several findings above, Red Ginger Extract is anti-arthritic and anti-inflammatory via the following mechanisms:

1. Inhibition on migration of human monocytes into inflammatory tissues
2. Selective inhibition of COX-2 leading to the suppression of PG production
3. Inhibition of NO production

Besides, it is also suggestive that Red Ginger Extract regulates the functions of osteoclast cells which posses similar properties to that of macrophages.

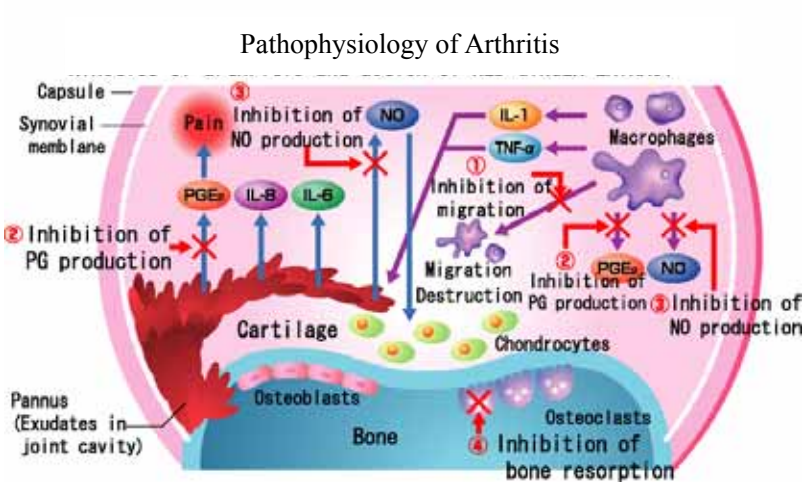


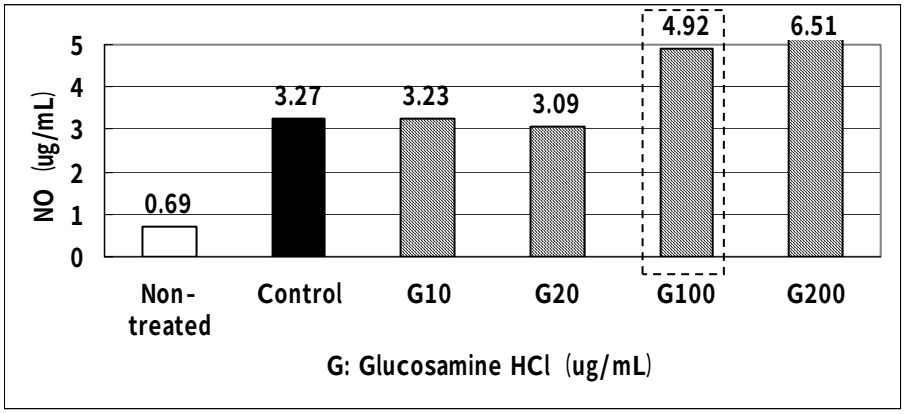
Fig. 13. The anti-arthritis effect of Red Ginger Extract – mechanism of actions & site of actions

A part of the data described above have published in the Journal
Shimoda H., Shan S.J., Tanaka J, Seki A, Seo J. W., Kasajima N., Tamura S, Ke Y., Murakami N. Anti-inflammatory properties of red ginger (*Zingiber officinale* var. rubra) extract and suppression of nitric oxide production by its constituents. *J. Med. Food* **13**, 1-7 (2010).

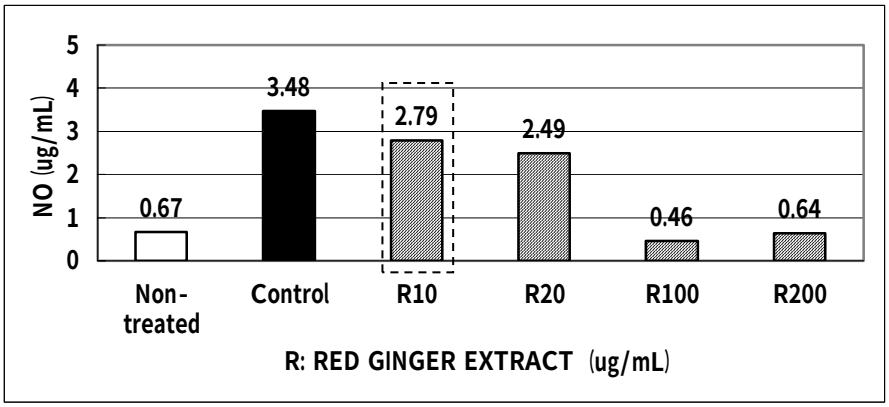
5) Synergistic inhibitory effect with glucosamine on NO production

We investigated synergistic effect of RED GINGER EXTRACT and glucosamine on NO production from macrophage. As shown Fig. 14 (upper), glucosamine HCl (10 and 20 µg/mL) did not suppress NO production and enhanced the production at 100 and 200 µg/mL. On the other hand, RED GINGER EXTRACT (10-200 µg/mL) suppressed NO production (Fig. 14, middle). Moreover, co-treatment of glucosamine HCl (100 µg/mL) and RED GINGER EXTRACT (10 µg/mL) more strongly suppressed NO production

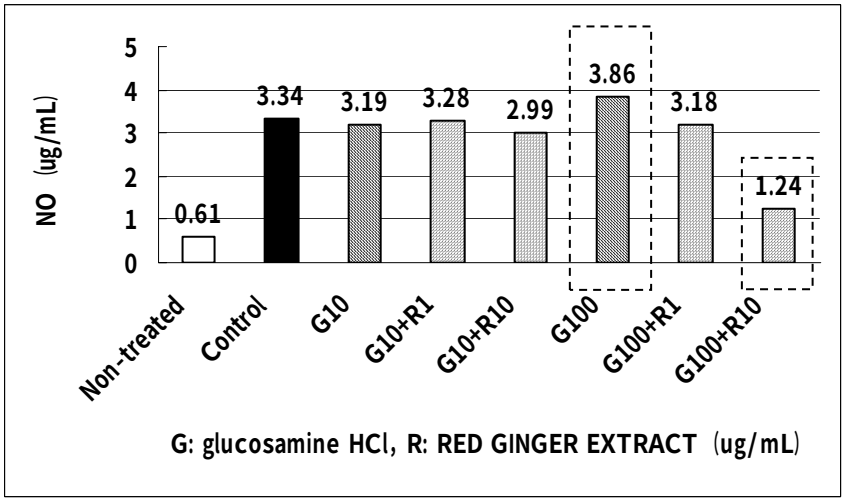
compared to the treatment of glucosamine (100 μg/mL) or RED GINGER EXTRACT (10 μg/mL) alone (Fig. 14, lower). Thus, the composition consist of 10 part of glucosamine and 1 part of RED GINGER EXTRACT was found to show synergistic suppressive effect on NO production.



Glucosamine HCl



RED GINGER EXTRACT



Co-treatment of RED GINGER EXTRACT and glucosamine

Fig. 14. Synergistic effect of RED GINGER EXTRACT and glucosamine on NO production

6) Enhancement of [6]-shogaol on adiponectin expression

Beside of arthritis, [6]-shogaol was reported to enhance adiponectin expression. TNF-alpha stimulated adiponectin production from adipocytes was enhanced by pre-treated with [6]-shogaol (Fig. 15).

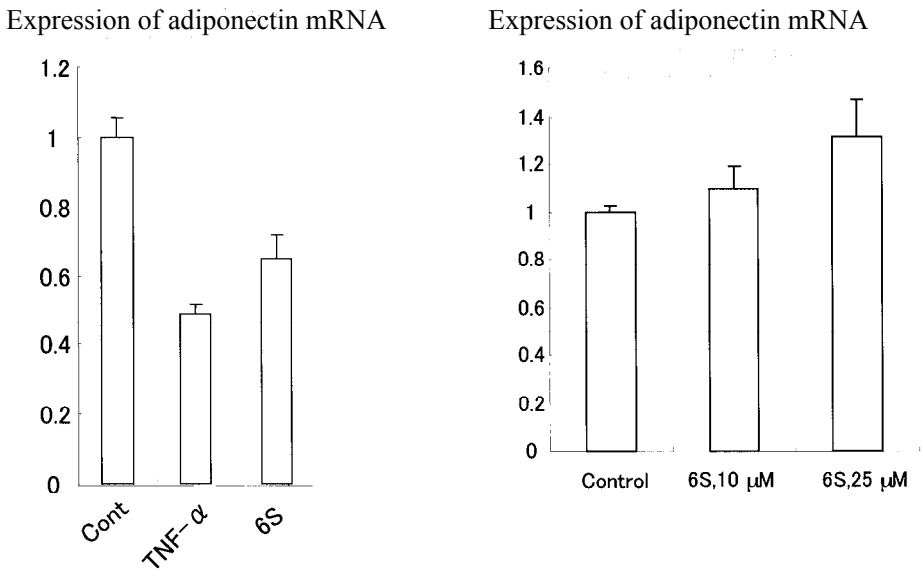


Fig. 15 . Effect of [6]-shogaol (6S) on adiponectin expression in 3T3-L1adipocytes stimulated by TNF-α

Right: TNF-α stimulated, Light: Non stimulation

4. The Effect of Red Ginger Extract on Inflammatory Parameters – Human Trial

Previous positive findings prompted an in-depth study on the effect of Red Ginger Extract on human. The effect of Red Ginger Extract on blood profile of arthritic patients was evaluated. Protocol of the study as follow:

No. of subjects: 9 male volunteers
Dosage: Red Ginger Extract 50mg/day
Duration: 28days
Observation: Blood Profile Screening on **blood protein, albumin, ratio A/G, hyaluronic acid, IgG, IgM, MMP-3 (matrix metalloprotein), blood platelet, ESR & CRP**

As tabulated in Table 2, hyaluronic acid, blood platelet and C-reactive protein (CRP) levels were lowered with treatment of Red Ginger Extract. Reduction in hyaluronic acid was especially significant ($p<0.05$) under the treatment of Red Ginger Extract as its level return to the healthy normal range. Hyaluronic acid is produced by synovial cells of the joint cavity and usually detected at high concentrations in synovial fluid of rheumatic patients. Upon destruction of joint cavity, hyaluronic acid penetrates into the blood stream resulting in elevated blood hyaluronic acid level. Red Ginger Extract is beneficial in rheumatoid arthritis with its hyaluronic acid lowering effect.

Table 2. The effect of Red Ginger Extract on Blood Parameters of Arthritic Patients

	Before ingestion	After ingestion	Standard value
Protein（g/dL）	7.1±0.3	7.2±0.4	6.5～8.3
Albumin（g/dL）	4.5±0.2	4.6±0.3	3.8～5.7
A/G	1.7±0.2	1.7±0.2	1.1～2.3
Hyaluronic acid（ng/mL）	**53.1±30.1**	**34.8±16.5** $p<0.05$	**<50**
IgG（mg/dL）	1166±229	1135±231	870～1700
IgM（mg/dL）	79.4±24.4	79.7±26.8	33～190
MMP-3（ng/mL）	88.5±23.0	91.2±22.1	36.9～121
Platelet（$\times 10^4$ /μL）	**23.4±4.7**	**19.5±5.9**	**13.1～36.2**
Erythrocyte sedimentation rate, 1h（mm）	4.9±2.3	5.1±2.4	1～7
Erythrocyte sedimentation rate, 2h（mm）	11.7±6.2	13.3±6.8	
CRP（ng/mL）	**927±1369**	**802±837**	**<1500**

Mean±S.D.（n=9）

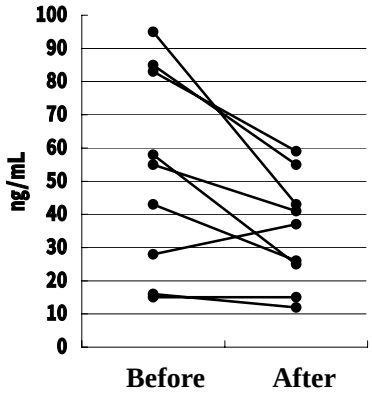


Fig. 16. The effect of Red Ginger Extract (oral) on blood hyaluronic acid

Shimoda H. 28 Anti-inflammatory properties of *Zingiber officinale* var. Rubra (*R*ed ginger extract). Arthritis, Edited by Bagchi D. and Moriyama H. pp.409-418 CRC Press. (2011).

5. Stability of RED GINGER EXTACT

(1) Thermostability

Evaluation on the thermostability of Red Ginger Extract (without binder) was conducted at 80°C & 100°C for 1 hour. As shown in Fig. 17, degradation of [6]-gingerol begins upon heating at 100°C due to decomposition of its essential oil components. Meanwhile, tannin degraded at 80°C and increased again at 100°C along with colour change (reddish brown) due to probable polymerization.

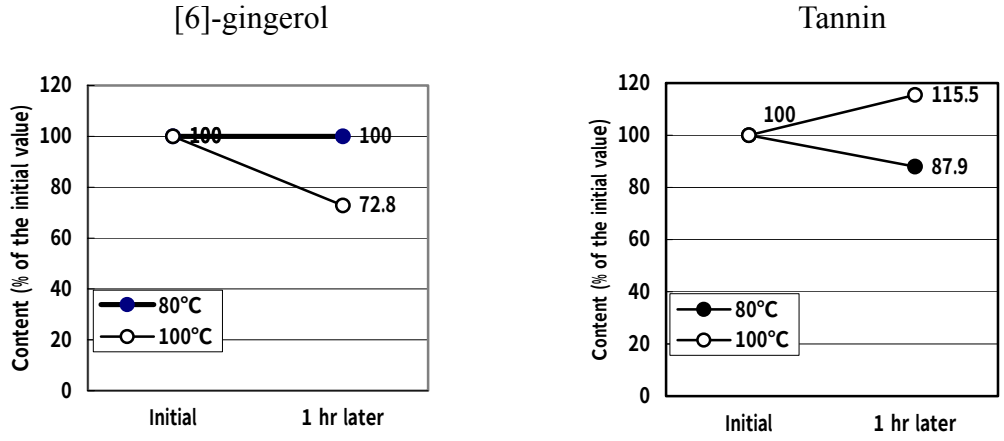


Fig. 17. Thermostability of RED GINGER EXTRACT

Changes on components of Red Ginger Extract in aqueous condition under commercial standard was studied. Red Ginger Extract-P 0.01% solution was prepared and heated according to the condition stipulated for sterilization of bottled beverages (i.e. 15 min at 85°C or 121°C). Results showed that content of [6]-gingerol and tannin were highly stable at high temperature.

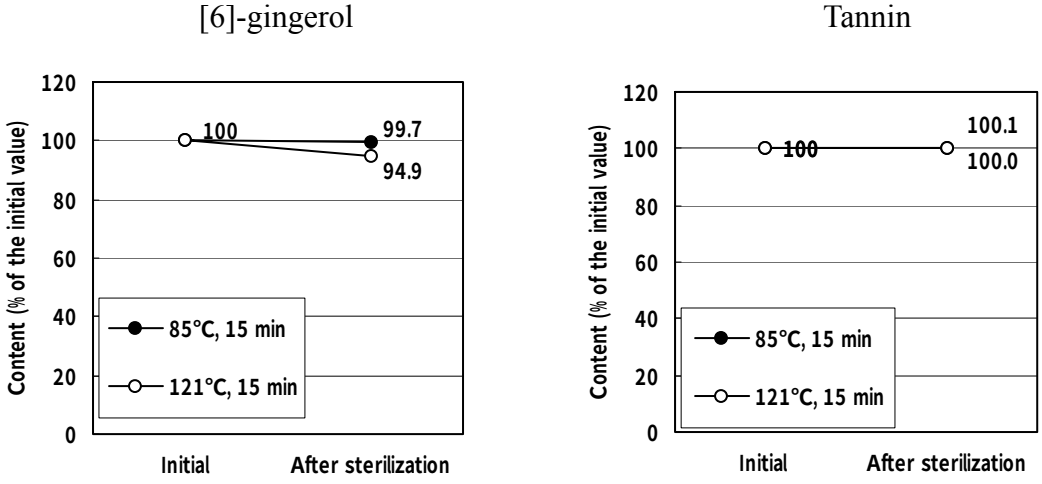


Fig. 18. Thermostability of RED GINGER EXTRACT-P in sterilizing condition

(2) pH stability

Evaluation on the pH stability of Red Ginger Extract was conducted. 0.01% and 0.1% of Red Ginger Extract-P solution was prepared and stored at different pH at 4°C for 3 days under darkness. Results showed that [6]-gingerol and tannin are highly stable ranges of pH 3 to pH 8.

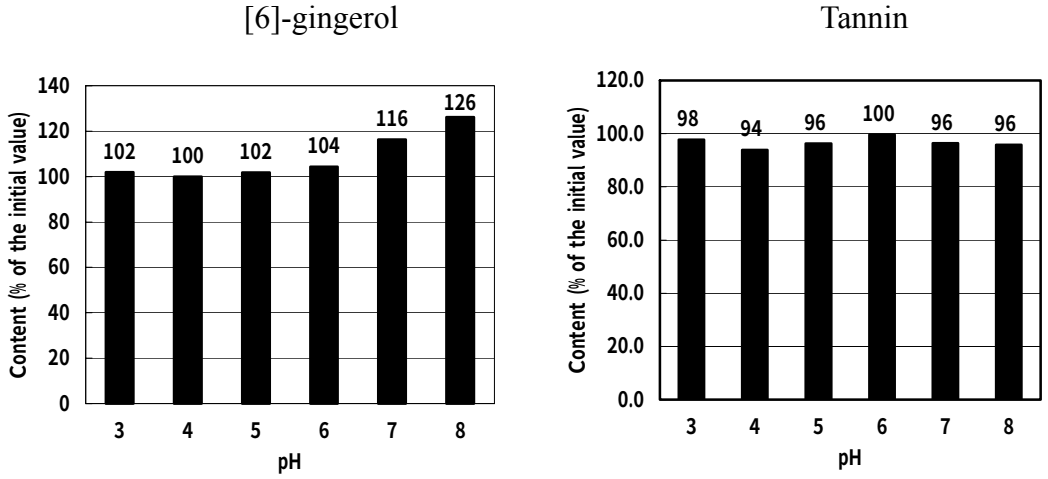


Fig. 19. pH stability of RED GINGER EXTRACT-P

(3) Photostability

Photostability of Red Ginger Extract was carried out using Red Ginger Extract-LC (containing Red Ginger Extract 1%) and stored under fluorescent light for 1 month. Results showed that there was no degradation of [6]-gingerol under fluorescent light.

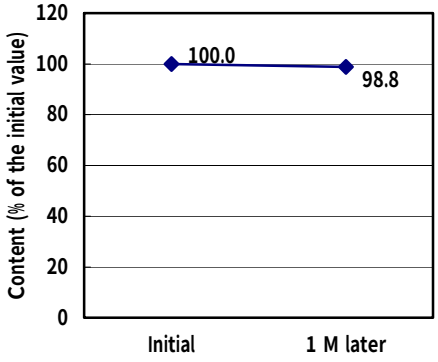


Fig. 20. Photostability of RED GINGER EXTRACT-LC

(4) Preservation stability

Study on the stability of Red Ginger Extract (without diluent) under preserved condition at room temperature started 8 months ago and still in progress. No significant degradation in [6]-gingerol observed to-date.

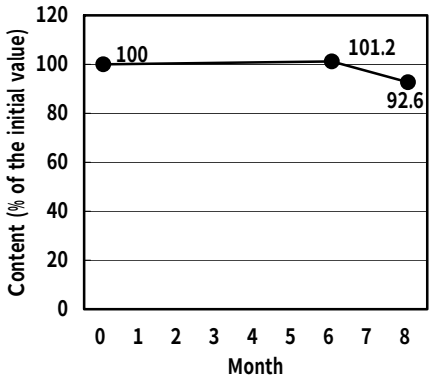


Fig. 21. Stability of preserved RED GINGER EXTRACT (without diluent)

6. Nutrition information (RED GINGER EXTRACT)

Description	RED GINGER EXTRACT-P	RED GINGER EXTRACT-WSP	Note	Analytical method
Water	5.4 g/100g	5.4 g/100g		Distillation method
Protein	37.4 g/100g	24.9 g/100g	1	Kjeldahl method
Fat	2.0 g/100g	1.3 g/100g		Soxhlet extraction method
Ash	16.6 g/100g	11.1 g/100g		Direct incineration
Carbohydrate	32.9 g/100g	53.5 g/100g	2	
Energy	311 kcal/100g	333 kcal/100g	3	Revised Atwater method
Dietary fiber	5.7 g/100g	3.8 g/100g		Enzymatic weight method
Sodium	120 mg/100g	80 mg/100g		Atomic absorption spectrophotometory

1. Nitrogen, protein, conversion factor: 6.25
2. Calculation：100 – (water+protein+fat+ash+dietary fiver)
3. Energy expression standard：protein 4, fat 9, sugar 4, dietary fiver 2

Test trustee：Farco Biosystem Co. Ltd
Date of analysis：April 26, 2006
Test No. REP03540

7. Safety of RED GINGER EXTRACT

(1) Residual agricultural chemicals

Red Ginger Extract (without binder) and Red Ginger Oil are complianced to the standards stipulated in the Food Sanitation Law by the Ministry of Health, Labour & Welfare for the 33 residual agricultural chemicals. None of the Residual Agricultural Chemicals is detected.

	Description	Result	Detection limit	Method
1	EPN	Not detected	0.5 ppm	GC-MS
2	EPTC	Not detected	0.1 ppm	GC-MS
3	Acephate	Not detected	0.5 ppm	GC-MS
4	Iprodione	Not detected	0.5 ppm	GC-MS
5	Etofenprox	Not detected	0.1 ppm	GC-MS
6	Etorimfos	Not detected	0.1 ppm	GC-MS
7	Cadusafos	Not detected	0.5 ppm	GC-MS
8	Chlorpyrifos	Not detected	0.05 ppm	GC-MS
9	Diethofencarb	Not detected	0.05 ppm	GC-MS
10	Cyhalothrin	Not detected	0.1 ppm	GC-MS
11	Thiobencarb	Not detected	0.05 ppm	GC-MS
12	Thiometon	Not detected	0.5 ppm	GC-MS
13	Tralomethrin	Not detected	0.1 ppm	GC-MS
14	Trifural;in	Not detected	0.02 ppm	GC-MS
15	Trichlorfos-methyl	Not detected	0.02 ppm	GC-MS
16	Parathion-methyl	Not detected	0.05 ppm	GC-MS
17	Bioresmethrin	Not detected	0.1 ppm	GC-MS
18	Pirimifos-methyl	Not detected	0.02 ppm	GC-MS
19	Fenarimol	Not detected	0.5 ppm	GC-MS
20	Fenobucarb	Not detected	0.5 ppm	GC-MS
21	Fenvalerate	Not detected	0.05 ppm	GC-MS
22	Furcythrinate	Not detected	0.1 ppm	GC-MS
23	Flutolanil	Not detected	0.5 ppm	GC-MS
24	Prothiofos	Not detected	0.05 ppm	GC-MS
25	Propamocarb	Not detected	0.5 ppm	GC-MS
26	Permethrin	Not detected	0.1 ppm	GC-MS
27	Pencycuron	Not detected	0.5 ppm	GC-MS
28	Boscalid	Not detected	0.1 ppm	GC-MS
29	Methiocarb	Not detected	0.1 ppm	GC-MS
30	Metribuzin	Not detected	0.1 ppm	GC-MS
31	Lenacil	Not detected	0.1 ppm	GC-MS
32	Dichlorvos	Not detected	0.1 ppm	GC-MS
33	Triflumizole (Include metabolite)	Not detected	0.05ppm	GC-MS

Test trustee：Kyusai Analytical Laboratory
Date of analysis：May 9, 2006
Test No. 2006032326-01,02

(2) Acute toxicity (LD_{50})

Safety of Red Ginger Extract was conducted according to the Pharmaceuticals Guidelines on Single-dose Toxicity Test. 2000mg/kg of Red Ginger Extract (maximum dosage without burden on animals) was given to fasting ICR male/female mice of 5-week old followed by close observation for 14 days. No fatal event and no abnormal changes observed in the weight of mice (compared to control). Similarly, no abnormal changes detected in organs of mice upon partial inspection conducted after the test. The LD_{50} (oral) of Red Ginger Extract on male/female mice is deduced to be >2,000mg/kg.

(3) Four-week repeated dose toxicity test

Toxicity on 28-day repeated dose was conducted on SD male rats. 50, 150 & 500mg/kg of Red Ginger Extract were given to rats without any restrictions on weight and test condition during the 28-day period. No abnormal changes observed in organs, weight and blood profile of rats at end of test.

(4) Mutagenicity

Ames Test was conducted and finding was Negative. Red Ginger Extract is non-mutagenic.

8. Recommended Dose of Red Ginger Extract

Recommended daily dose: 15 to 20 mg of RED GINGER EXTRACT-P.

9. Crude Drug Equivalent

1g of RED GINGER EXTRACT-P is equivalent to approximately 44 g of raw red ginger. Recommended daily dose is equivalent to 660 to 880 g of raw red ginger.

10. Commercial application

	Application	Claims	Example
Foods	Relief of joint pain	Prevention of arthropathy (knee arthritis, rheumatism, etc)	Beverages, hard & soft capsule, tablets, candies, chewing gum, chocolates, wafers, jellies, etc
Cosmetics	Anti-aging cosmetics		Body lotion, body gel, etc

11. Packaging

RED GINGER EXTRACT-P (powder, for food), -WSP (water-soluble powder, for food)

5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard

RED GINGER EXTRACT-PC (powder, for cosmetics), -WSPC (water-soluble powder, for cosmetics)

5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard

RED GINGER EXTRACT –LC (water-soluble liquid, for cosmetics)

5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard

12. Storage

Store in cool, dry dark place.

13. Expression

<Food>

Red ginger extract-P, -WSP
Expression: Red ginger extract, cyclodextrin

<Cosmetics>

Red ginger extract-PC

INCI name：Zingiber Officinale (Ginger) Rhizome Extract, Cyclodextrin

Red ginger extract-WSPC

INCI name：Cyclodextrin, Zingiber Officinale (Ginger) Rhizome Extract

Red ginger extract-LC

INCI name：Butylene Glycol, Water, Zingiber Officinale (Ginger) Rhizome Extract

14. Certification

Red Ginger Extract-PC has been obtained ECOCERT.

PRODUCT STANDARD

PRODUCT NAME

RED GINGER EXTRACT-P

Food

This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous ethanol. It guarantees minimum 6.0% [6]-gingerol and [6]-shogaol, and 1.5% tannin. Moreover a peak of 3*R*,5*S*-[6]-gingerdiol can be observed in HPLC chromatogram of this product.

Appearance	Pale yellow or yellowish brown powder with unique smell and pungent flavor.	
[6]-gingerol and [6]-shogaol	Min. 6.0 %	(HPLC)
Tannin (Equivalent of procyanidin B_2)	Min. 1.5 %	(Vanillin・HCl method)
3*R*,5*S*-[6]-gingerdiol	A pee is detectable	(HPLC)
Loss on drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 ℃, 2 h)
Purity test		
（1）Heavy metals (as Pb)	Max. 30 ppm	(Sodium Sulfide Colorimetric Method)
（2）Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Red ginger extract	50 %
Cyclodextrin	50 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

RED GINGER EXTRACT-WSP

Food

This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous ethanol. It guarantees minimum 3.0% [6]-gingerol and [6]-shogaol, and 0.5% tannin. Moreover, a peak of 3*R*,5*S*-[6]-gingerdiol can be observed in HPLC chromatogram of this product.

Appearance	Yellowish white powder with slightly unique smell and pungent flavor.	
[6]-gingerol and [6]-shogaol	Min. 3.0 %	(HPLC)
Tannin (Equivalent of procyanidin B_2)	Min. 0.5 %	(Vanillin・HCl method)
3*R*,5*S*-[6]-gingerdiol	A peek is detectable	(HPLC)
Loss on drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 ℃, 2 h)
Purity test		
（1）Heavy metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
（2）Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Red ginger extract	33 %
Cyclodextrin	67 %
Total	100 %