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Effects of Several Natural Extracts on Experimental Gouty Arthritis in Rats

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Abstract. Gouty arthritis results from monosodium urate (MSU) crystal deposition in joint tissues. The deposited MSU crystals induce an acute inflammatory response which leads to damage of joint tissue, with edema and erythema of joints and severe pain. This paper investigated the anti-gouty arthritis mechanism of four natural substances, astaxanthin, apple polyphenol, gypenoside and puerarin, through the methods of measuring paw volume of rats, assaying the level of Myeloperoxidase (MPO) activity, IL-1β and IL-8 in serum. Collectively, this study demonstrates that astaxanthin may be of value in treatment of MSU crystal-induced gouty arthritis through its anti-inflammatory activities.

Keywords: Gouty arthritis, Astaxanthin, Apple polyphenol, Gypenoside, puerarin, MPO, IL-1β, IL-8.

1. Introduction

Gout appears to be a common metabolic disorder affecting humans, presenting as an acute and painful inflammatory arthritis, mostly of the first metatarsophalangeal joint, and occurs suddenly. And the biochemical basis of gout is hyperuricemia, leading to the deposition of monosodium urate (MSU) crystals in joints and kidneys, and also resulting in gouty arthritis and uric acid nephrolithiasis [1, 2]. Gouty arthritis, suffered by a growing number of people, is usually an extremely painful attack with a rapid onset of joint inflammation. And it is characterized by marked swelling, severe pain and especially the disorder of joint movement. And gouty arthritis is the inflammation caused by deposition of monosodium urate (MSU) in the joints, consequently resulting the acute inflammatory response [3]. Current treatments to gouty arthritis during an acute attack include colchicine, corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs). Although these agents are generally effective, they also present adverse side effect in patients who have pre-existing renal, cardiovascular and gastrointestinal diseases. Therefore, there is a need for new therapies to provide effective pain relief in these patients with difficult-to-treat Gouty Arthritis [4]. And the current research to develop medicines for gouty arthritis has focused on the natural substances [5].

In this paper, we will investigate the anti-gouty arthritis mechanism of four natural products, astaxanthin, apple polyphenol, gypenoside and puerarin. Astaxanthin, 3,3'-dihydroxy-β, β-carotene-4.4'-dione, is a carotenoid which has shown anti-oxidant and anti-inflammatory activities. The anti-inflammation mechanism of astaxanthin is related to inhibition of lipid peroxidation and prostaglandin E2. Apple polyphenol is an important biological substance which has benzene ring and hydroxyl group, the strong

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antioxidant. Gynostemma pentaphyllum is a perennial creeping vine that grows mainly in the countryside of Southern China, Taiwan, Japan and Korea. Gypenoside is the main active constituent of gynostemma pentaphyllum, which has the same structure type with ginsenoside. Traditional Chinese doctors use G. pentaphyllum to treat hypertension, hyperlipidaemia and lung inflammation. Modern research found that G. pentaphyllum has additional pharmacological effects that are useful for treating hyperlipidaemia, hepatitis, gastric ulcers and cancer as well as for regulating blood sugar and enhancing immunity [6]. Puerarin is a major active product extracted from the root of Pueraria, a traditional Chinese herb, and possesses anti-oxidative and anti-inflammatory activities [7]. And it is envisaged that the baseline information provided in this research will be useful and applicable for future study works aiming towards exploiting the plants nutraceutical potentials.

2. Materials and Methods

2.1. Animals

Wistar rats (200±20g) of SPF families were purchased from Beijing Vital River Laboratories (Beijing, China) and were housed one week to adapt to their environment before used for experiments. All rats were maintained on a 14/10-hr light/dark cycle at a constant temperature of 22±2℃ and a humidity of 40-60% with free access to food and water for the duration of the study. All the experiments and animal care were performed strictly in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation.

2.2. Natural Products and Reagents

Apple polyphenol (purity≥80%) was purchased from Tianjin Jian feng Natural Product Co.Ltd (Tianjin, China). Astaxanthin was purchased from BioReal (Sweden). Gypenoside (purity≥80%) and puerarin (purity≥80%) were purchased from Shanghai Seni Pharma-Tech Co.Ltd (Shanghai, China). Commercial kit used for the determination of Myeloperoxidase (MPO) was purchased from Jiancheng Biotech (Nanjing, China). Uric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Synthesis of Monosodium Urate Crystals

MSU crystals were synthesized by carrying out the procedure reported by Ortiz-Bravo et al [8]. A mixture containing 1g of uric acid, 6 ml of 1 M NaOH and 194 ml of distilled water were heated. HCl (1 N) was then added to maintain the mixture at pH 7.2. The mixture was stirred slowly at room temperature and then stored at 4℃ for 24 h. At last the MSU crystals in the mixture were dried after washing and then suspended in sterile saline (100 mg/ml).

2.4. Experimental Design and Treatment Schedule

The natural products of astaxanthin, apple polyphenol, gypenoside and puerarin were given by intragastric administration once daily for 14 consecutive days. And at the 11^{th} day, the model of gouty arthritis could be made in rats 1 h after drug administration through injecting MSU in right ankle joint. In the study, forty-eight rats were divided at random into six groups. Group 1 served as control. In group 2, gouty inflammation was induced by ankle joint cavity injection of 50 μl monosodium urate crystal suspension into the right ankle joint. And group 3-6 comprised monosodium urate crystal-induced rats treated with astaxanthin, apple polyphenol, gypenoside and puerarin respectively at a daily dose of 200 mg/kg bodyweight.

- **Sample Collection**

Blood samples were collected from rats femoral artery at 72 h after injection of MSU. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 4℃ to obtain the serum. The serum was stored at -20℃ until use.

- **Assessment of the Joint Swelling**

The ankle joint volume was measured before injection of monosodium urate and then at 10, 24, 48 and 72 h after injection. Then the joint swelling rate (Joint edema rate = (joint swelling after modeling / joint swelling before modeling -1)×100) could be calculated.

- **Determination of Neutrophil Infiltration**

To evaluate the possible cellular infiltration induced by MSU, myeloperoxidase (MPO) activities were used as an index of neutrophil accumulation. And the MPO was assayed in serum by kits.

- **Enzyme Immunoassay of Interleukin-1β (IL-1β) and Interleukin-8 (IL-8)**

IL-1β and IL-8 were measured by enzyme-linked immunosorbent assay according to the manufacture's protocol.

2.5. Statistical Analysis

Statistical data are expressed as the means±standard deviation (S.D.). The statistical analyses were performed using the SPSS program (SPSS 11.5, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to determine the significant differences between the groups. Probability values <0.05 were considered significant.

3. Results

3.1. Effects of Four Materials on Joint Swelling in MSU Crystal-induced Gouty Arthritic Rats

As shown in Table 1, the joint swelling rate of model group was increased singnificantly compared with control group at 10 h, 24 h, 48 h and 72 h after injection. And joint swelling rate of each administration group was significantly lower than the model group at 24h after modeing. The astaxanthin group's joint swelling was the smallest at 10h, 24h, and 72h respectively after injecting MSU. The result indicated that astaxanthin effectively prevented the development of gouty arthritis swelling, and implied that astaxanthin possesses an anti-inflammatory effect, which could provide relief for the gouty arthritis.

Table 1 Effects of astaxanthin, apple polyphenol, gypenoside and puerarin on joint swelling[1,2)] in MSU crystal-induced gouty arthritic rats

Groups	10h	24h	48h	72h
Control	5.52±3.30	3.42±2.81	1.15±2.19	0.61±1.80
Model	33.39±3.34##	33.30±4.26##	15.85±3.45##	11.85±3.49#
Astaxanthin	27.23±4.00	22.02±2.83*	11.50±2.88	5.99±1.95
Apple polyphenol	32.14±2.40	23.36±2.80*	13.32±2.37	10.59±2.54
Gypenoside	33.24±3.23	22.37±3.01*	9.13±2.72	10.93±2.97
Puerarin	28.17±3.96	22.95±3.14*	9.67±3.06	10.31±4.11

1)Joint edema rate (%) = (joint edema after modeling / joint edema before modeling -1)×100;
2)Values are mean ± SD (n=8);
* P<0.05, compare with model; # P<0.05, compare with control; ## P<0.01, compare with control.

3.2. Effects of Four Materials on the Activity of MPO in MSU Crystal-induced Gouty Arthritic Rats

Fig. 1 presents the activity of MPO in serum. The level of MPO activity in model group was significantly higher than that in control group, which indicated that the gouty arthritis resulted in inflammation in rats. And the MPO activity in astaxanthin and apple polyphenol groups were significantly decreased compared with model group. So astaxanthin and apple polyphenol could restrain the MPO activity and played an effective role in inhibition of inflammation.

3.3. Effects of Four Materials on Interleukin-1β and IL-8 in MSU Crystal-induced Gouty Arthritic Rats

To examine the anti-gouty inflammation potential and effective mechanism of the four materials against inflammation, the levels of IL-1β and IL-8 were determined and the results were shown in Fig 2. The level of IL-1β (Fig 2 A) in model group was significantly higher than that in control group. Decreased levels of IL-1β were found in astaxanthin, gypenoside and puerarin groups compared with model. Furthermore the astaxanthin group was significantly decreased. The result implied that astaxanthin possesses an anti-inflammatory effect, which provides a better inhibition of IL-1β.

Fig 2 B shows the level of IL-8 in serum. The level of IL-8 in model group was significantly higher than that in control group, which indicated that gouty arthritis induction treatment using MSU in rats could result

in the elevated level of IL-8 and accelerate the inflammation. And the level in astaxanthin group was lower than other material groups, but no significance.

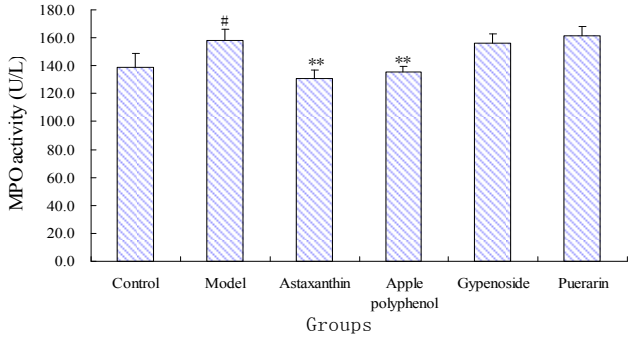

Fig. 1: Effects of astaxanthin, apple polyphenol, gypenoside and puerarin on the activity of MPO in MSU crystal-induced gouty arthritic rats. Values are expressed as mean±S.D, n=8. ** P<0.01, compared with model, # P<0.05, compared with control.

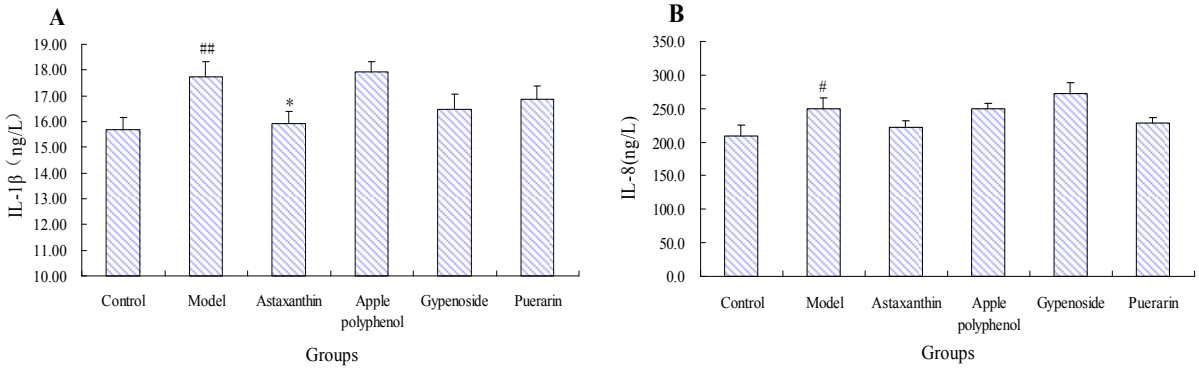

Fig. 2: Effects of astaxanthin, apple polyphenol, gypenoside and puerarin on IL-1β (A) and IL-8 (B) in MSU crystal-induced gouty arthritic rats. Values are expressed as mean±S.D, n=8. * P<0.05, compare with model, # P<0.05, compare with control, ## P<0.01, compare with control.

4. Discussion

Gouty arthritis is characteristically acute inflammatory reaction which occur in response to articular deposits of monosodium crystals, and associated with edema and erythema of joints, together with severe pain [9]. The primary pathologic hallmark of gouty arthritis is neutrophil influx into the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytose monosodium urate crystals and release mediators, that are chemotactic and amplify the inflammatory reaction.

During inflammation, MPO is released, and its measurement in systemic circulation may be used as an index of neutrophil infiltration [10]. And in this stage, some reactive intermediates formed by MPO-catalyzed reactions may modify signaling mediators, and leading to alterations in cellular signaling [11]. In the study the MPO activity in gouty arthritis rats was significantly higher than the control group, which implied that the massive influx of neutrophils in acute inflammation, and astaxanthin and apple polyphenol can significantly alter the above change by reducing the activity of MPO.

The mechanism of how MSU increased IL-1β production was not known until the seminal work of Martinon et al [12]. It was demonstrated that the inflammatory effects of the MSU crystals worked through the NOD-like receptor (NLR) proteins inflammasome.

Interleukin-8 (IL-8) is an important mediator of the inflammatory response in serious bacterial infections and chemotactic agent of neutrophils and lymphocytes [13] IL-8 is produced by multiple cell types, including monocytes, endothelial cells, fibroblasts, lymphocytes, neutrophils, keratinocytes, epithelial cells,

some tumor cells and so on [14]. In this study, significantly increasing levels of IL-1β and IL-8 indicated the inflammation and injury in MSU crystal-induced rats. And astaxanthin performed well in reversing the elevations of IL-1β and IL-8.

To conclude, the results of the present study suggest that astaxanthin treatment at the dosage of 200 mg/kg bodyweight possesses anti-gouty inflammation. Firstly, it can significantly reduce the joint swelling. Then the PMN's recruitment to the site of inflammation are also inhibited through the decreased chemotactic factor IL-8. Astaxanthin can significantly inhibit the inflammatory cytokine IL-1β. However, further pharmacological evidences would be required to establish the mechanism of the action of the material.

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6. References

[1] E.P. Sabina, M. Rasool. An in vivo and in vitro potential of Indian ayurvedic herbal formulation Triphala on experimental gouty arthritis in mice. *Vascular Pharmacology*. 2008, **48**: 14-20.

[2] Pascal Richette, Thomas Bardin. Gout. *Lancet*. 2010, **375**: 318-328.

[3] Jieun Lee, Jeongmi An, Hee-Jin Yang, et al. Reparatory and Preventive Effects of Oriental Herb Extract Mixture (OHEM) on Hyperuricemia and Gout. *Food Sci. Biotechnol*. 2010, **19** (2): 517-524.

[4] Naomi Schlesinger1, Marc De Meulemeester, Andrey Pikhlak, et al. Canakinumab relieves symptoms of acute flares and improves health-related quality of life in patients with difficult-to-treat Gouty Arthritis by suppressing inflammation: results of a randomized, dose-ranging study. *Arthritis Research & Therapy*. 2011, **13**: 53.

[5] Alexander Gosslau, Shiming Li, Chi-Tang Ho, Kuang Yu Chen, Nancy E. Rawson. The importance of natural product characterization in studies of their anti-inflammatory activity. *Mol. Nutr. Food Res*. 2011, **55**: 74-82.

[6] Chian-Jiun Liou, Wen-Chung Huang, Ming-Ling Kuo, et al. Long-term oral administration of Gynostemma pentaphyllum extract attenuates airway inflammation and Th2 cell activities in ovalbumin-sensitized mice. *Food and Chemical Toxicology*. 2010, **48**: 2592-2598.

[7] Cheng Xiao, Jian Li, Xinxin Dong, et al. Anti-oxidative and TNF-α suppressive activities of puerarin derivative (4AC) in RAW264.7 cells and collagen-induced arthritic rats. *European Journal of Pharmacology*. 2011, **666**: 242-250.

[8] Ortiz-Bravo E, Sieck MS, Schumacher HR Jr. Changes in the proteins coating monosodium urate crystals during active and subsiding inflammation. *Arthritis Rheum*. 1993, **36**: 1274-1285.

[9] Evan Prince Sabina, MahaboobKhan Rasool, Lazar Mathew. 6-Shogaol inhibits monosodium urate crystal-induced inflammation-An in vivo and in vitro study. *Food and chemical toxicology*. 2010, **48**: 229-235.

[10] Kamyar Kalantar-Zadeh, Marie-Luise Brennan, Stanley L.Hazen. Serum Myeloperoxidase and Mortality in Maintenance Hemodialysis Patients. *American Journal of Kidney Diseases*. 2006, **48** (1): 59-68.

[11] Lukas Kubala, Kara R.Schmelzer, Anna Klinke, et al. Modulation of arachidonic and linoleic acid metabolites in myeloperoxidasedeficient mice during acute inflammation. *Free Radical Biology & Medicine*. 2010, **48**: 1311-1320.

[12] Martinon, F., Tschopp, J. Inflammator caspases and inflammasomes: master switches of inflammation. *Cell Death and Differentiation*. 2007, **14**: 10-22.

[13] Kjell Tullus, Omar Fituri, Lars G.Burman, Bengt Wretlind, et al. Interleukin-6 and interleukin-8 in the urine of children with acute pyelonephritis. *Pediatric Nephrology*. 1994, **8** (3): 280-284.

[14] M.S. Angst, J. D. Clark, B. Carvalho, et al. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: A microdialysis study. *Pain*. 2008, **139**: 15-27.