Food Sci. Biotechnol. Vol. 16, No. 1, pp. 43 ~ 48 (2007)

Food Science and Biotechnology

Suppressive Effects of Potato (*Solanum tuberlosum*) on Type II Collagen-Induced Arthritis in DBA/1J Mice

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Abstract Collagen-induced arthritis (CIA) is a model for some types of human autoimmune rheumatoid arthritis (RA). In this study, we examined whether ethanol extract of potato (*Solanum tuberosum*) is efficacious against CIA in mice. Potato extracts (100 and 200 mg/kg) were orally administered to DBA/1J mice once daily for 49 day after initial immunization with type II collagen. Clinical assessment of disease and measurement of paw edema were conducted throughout the study. The production of CIA-related rheumatoid factor, anti-type II collagen antibody, and cytokines were examined in DBA/1J mice. Serum levels of AST, ALT, creatinine, and lipids were measured, and antioxidant enzyme activity in the spleen was also determined. The arthritis score and paw edema were markedly suppressed in the groups treated with potato extract. Levels of rheumatoid factor, anti-type II collagen antibody, interleukin (IL)-1, IL-6, LDL-cholesterol, and malondialdehyde in sera were also reduced by potato extract treatment. The activities of glutathione peroxidase and glutathione reductase were increased in the spleens of CIA mice treated with potato extract. These findings suggest that potato extract has suppressive effects on type II collagen-induced arthritis, an animal model for human RA.

Keywords: *Solanum tuberosum*, collagen-induced arthritis, DBA/1J mouse

Introduction

Rheumatoid arthritis (RA), an autoimmune disease of unknown origin, is associated with inflammation of the joints and a variety of systemic problems. The rheumatoid joint shows an inflammatory cell infiltrate comprised of neutrophils, macrophages, T and B lymphocytes, and dendritic cells (1). In the synovitis of rheumatoid arthritis, leukocytes that have migrated into synovial tissue generate cytokines (e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6), which enhance inflammation (1). The overproduction of cytokines along with other factors results in cartilage destruction, bone erosion, and remodeling of joint structures (2, 3). RA has been associated not only with free radical formation but also with low antioxidant status (4). Lipid peroxidation is considered to be a critical mechanism for the injury that occurs during RA (5). In a study conducted by Kalavacherla *et al.* (6), the concentrations of plasma malondialdehyde (MDA) in cases of RA were significantly higher than the concentrations estimated in controls. Antioxidant enzymes prevent cells from free radical-mediated disturbances by scavenging oxygen reactive species and products of lipid peroxidation. The superoxide dismutase (SOD) enzyme catalyses dismutation of the superoxide anion into hydrogen peroxide, while glutathione peroxide (GPx) detoxifies hydrogen peroxide and converts lipid hydroperoxides into non-toxic alcohols. During the reduction of such peroxides, oxidized glutathione (GSH) is produced, which is then reduced by glutathione reductase (GR) to regenerate GSH (7).

Type II collagen-induced arthritis (CIA) is induced in susceptible strains of mice, e.g., DBA1/J, by immunization with bovine collagen type II in complete Freund's adjuvant. The development of CIA is thought to depend on T cells, and disease susceptibility is linked to the major histocompatibility complex (MHC) region (8). Following T cell activation an inflammatory cascade is triggered involving T cells, macrophages/monocytes, B cells, and activated synoviocytes (1). Since CIA in mice is well known to have both clinical and histological similarities to human RA (9), these models have been widely used to evaluate anti-arthritic drugs (10). Anti-inflammatory drugs transiently reduce symptoms, yet the disease progresses over time. In addition, non-steroidal anti-inflammatory drugs induce gastric or hepatic toxicity (11), which limits their long-term usefulness. Therefore, over the last few years many research studies have focused on edible plants with therapeutic properties. One of the most attractive approaches to disease prevention involves the use of specific nutrients to protect tissue against toxic injury and degenerative diseases.

Potato (*Solanum tuberosum* L.) is one of the most widely consumed vegetables in the world. The juice of raw potato is used to improve the quality of life in patients suffering from rheumatoid arthritis and pain in Korea. Despite the use of the raw juice of peeled potatoes to enhance recovery from RA, there have been few studies to establish a scientific basis for such claimed benefits. The health benefits of potato consumption seem to be related, at least partly, to the presence of antioxidant phenolic metabolites. Moreover, potatoes are known to contain alkaloids. Steroidal glycoalkaloids are naturally-occurring, secondary plant metabolites found in a number of foods including potatoes, tomatoes, and eggplants (12). Although they are reported to be potentially toxic, glycoalkaloides and their hydrolysis products without the carbohydrate side chain (aglycons) also have beneficial effects. These effects include lowering of cholesterol (13), protection against infection by *Salmonella typimurium* (14), cancer

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Received June 27, 2006; accepted November 20, 2006

prevention (15), and potentiation of general anesthetics that act by inhibiting cholinesterage (16). They can also act as a malaria vaccine (17). Solanidine, but not the parent glycoalkaloids, exhibited estrogenic activity in an *in vitro* assay (18). In our previous study, potato extract showed anti-nociceptive and anti-inflammatory effects in acute and subacute animal models (19, 20). To investigate the anti-inflammatory and anti-oxidant role of potato extract in rheumatoid arthritis, we have examined the effects of potato extract on the RA parameters, oxidation products, and antioxidant enzymes in DBA/1J mice with CIA.

Materials and Methods

Preparation of extract Potatoes were obtained from a market in Seoul, Korea. The potatoes were peeled off to a depth of almost 1 mm with a vegetable peeler. The freshly blended potatoes were immediately extracted in EtOH 3 times. The extract was filtered, concentrated with a rotary evaporator at temperatures below 50°C, and freeze dried (yield: 2.2%w/w).

Animals Female DBA/1J mice, aged 6 weeks, were purchased from Japan SLC Inc. (Shizuoka, Japan). Animals received food and water *ad libitum* and were acclimatized to standard laboratory conditions (25±3°C, 55 ±5% humidity and 12 hr light/dark cycle) for 7 days. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy of Science, Bethesda, MD, USA) (21).

Induction of CIA and the experimental protocol Type II collagen (Sigma, St. Louis, MO, USA) was dissolved overnight at 4°C in 50 mM acetic acid to 2 mg/mL. This solution was then emulsified in an equal volume of Freund's complete adjuvant (Sigma) in an ice-cold water bath. DBA/1J mice were immunized with 0.1 mL of this emulsion by intradermal injection into the right hindpaw. Mice were given booster shots 21 day later. Potato extract (100 and 200 mg/kg) was orally administered once daily for 49 day after initial immunization with type II collagen. Fifty days after the initial injection, animals were sacrificed by decapitation. Blood was collected by heart puncture and centrifuged at 3,000×g for 15 min. Isolated spleen tissue was removed and prepared as described by Hong *et al.* (22). The chilled exsanguinated tissue was then cut into approximately 50- to 100-mg portions on ice and stored separately in plastic vials at -70°C. Homogenates from these samples were prepared after the addition of 1.0 mL phosphate buffer per 100 mg of tissue (22). Protein preparations were measured by the method of Bradford using a protein assay kit (Bio-Rad Lab., Hercules, CA, USA).

Assessment of arthritis Clinical severity was determined by palpation and observations of joint properties and inflammation of surrounding tissue. Arthritis was assessed on a scale of 0-3, using a previously published scoring system (23): 0=normal, 1=slight swelling and/or erythema of the fingers, 2=pronounced edematous swelling, and 3= joint rigidity with edematous swelling or joint ankylosis. Scores of 1 and 2 mainly reflect reversible edematous inflammation, but a score of 3 reflects irreversible components like established joint ankylosis. Edema in the hind paw was measured before the initial injection of type II collagen and after booster injections, using a dial thickness gauge. Body weight, clinical score, and hindpaw swelling were monitored weekly over a period of 49 day.

Biochemical analysis in serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, as well as serum creatinine levels, were assayed using a commercial kit (YD Diagnostics, Seoul, Korea). Rheumatoid factor (RF) level was assayed using a mouse RF enzyme-linked immunosorbent assay (ELISA) kit (Alpha Diagnostic, San Antonio, TX, USA). Anti-type II collagen IgG was measured by ELISA (Chondrex, Redmond, WA, USA). Cytokine levels were determined using ELISA kits for murine TNF-α, IL-1β, and IL-6 (R& D Systems, Minneapolis, MN, USA). Serum concentrations of triglyceride (TG), total cholesterol, and high density lipoprotein (HDL) cholesterol in were determined enzymatically using commercially available kit reagents (Mannheim-Boehringer, Mannheim, Germany). Low density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula: LDL cholesterol = total cholesterol - HDL cholesterol - triglyceride / 5 (3). Lipid peroxidation (as MDA) levels were measured with the thiobarbituric acid reaction by the method of Placer *et al.* (24). A standard curve was obtained with a known amount of 1.1.3.3.-tetraethoxypropane, using the same assay procedure.

Enzyme assay in the spleen of DBA/1J mouse Activity of superoxide dismutase (SOD) was determined according to the method of Marklund and Marklund (25), GPx was measured according to Flohe and Gunzler (26), and GR activity was assayed by the method of Carlberg and Mannervik (27).

Statistical analysis The results are expressed as mean ± SEM (n=7). Statistical significance was determined by analysis of variance and subsequent Duncan's multiple range test and by Kruskal Wallis test, for clinical score (p <0.05). The analysis was performed using SAS statistical software.

Results and Discussion

Mice with CIA develop a systemic autoimmune disease characterized by swelling of the hindpaws, degradation of the articular cartilage, circulating immune complexes, and rheumatoid factors, as well as autoantibody formation. Both RA and CIA are dependent on cytokines such as IL-1β and TNF-α to drive the inflammation and joint destruction (28). Because RA and CIA share many of the same underlying immunopathologic mechanisms, the murine CIA model is widely used to study disease mechanisms and potential therapies for RA. To investigate the effect of potato extract on established CIA, mice were treated daily with potato extract (100 and 200 mg/kg) or vehicle for 49 days. Animals were monitored weekly by measuring body weight, hindpaw thickness, and visual evaluation for the

appearance of clinical signs of arthritis. Over the study period, the body weights of mice steadily increased and did not show significant difference among groups (data not shown).

Symptoms developed after adjuvant treatment was not restricted to local inflammatory response and distal joint destruction. Tissue damage was measured clinically by assay of enzyme activity in sera and tissues. To examine whether potato extract had any toxicity *in vivo*, AST, ALT, and creatinine levels were measured in serum. The hepatoprotective effect was correlated with decreases in both the AST and ALT values. The increase in aminotransferase levels in serum may be due to the release of enzymes from the cells of damaged organs. As shown in Table 1, treatment with potato extract did not cause any increase in AST and ALT activities. Moreover, the serum creatinine level, used to assess kidney function, did not show significant difference among the groups. These results indicated no liver or kidney failure in the mice treated with potato extract. The observed increase in serum aminotransferase was due to liver impairment, a normal feature of adjuvant arthritis (29). A marked increase in the tissue and serum enzyme (AST and ALT) levels was observed in arthritic rats (30). Extrahepatic adjuvant treatment invades the local lymph nodes to cause proinflammatory cytokine (e.g., TNF-α, IL-6, and IL-1β) release, which consequently triggers systemic responses (e.g., liver, adrenal, stomach, and articular joints) (31, 32). Hung *et al.* (33) found that subcutaneous caudal administration of adjuvant to rats changed their liver architecture, biochemistry, and drug disposition/metabolism.

Clinical score peaked at day 14 and the changes in hindpaw swelling were most pronounced in the immunized group at day 21 (Fig. 1). The parameters for assessment of arthritis in CIA animals were significantly different from those of non-immunized animals throughout the experiment. The arthritic score for mice given potato extract (100 and 200 mg/kg) was significantly ($p<0.05$) lower than that in the CIA group (Fig. 1A). Measurements of paw swelling in the animals also showed potato extract to be effective (Fig. 1B). Taken together, these results demonstrate that potato extract, at doses of 100 and 200 mg/kg/day, had a preventive effect on CIA. Clinical scores mainly reflect joint conditions such as ankylosis. Therefore, this data may indicate that potato extract ameliorates edematous swelling, as well as already established joint ankylosis.

Rheumatoid factor (RF) was described as Immunoglobulins (IgGs) in rheumatoid arthritis (34). It was later demonstrated that RFs were IgM molecules reactive with

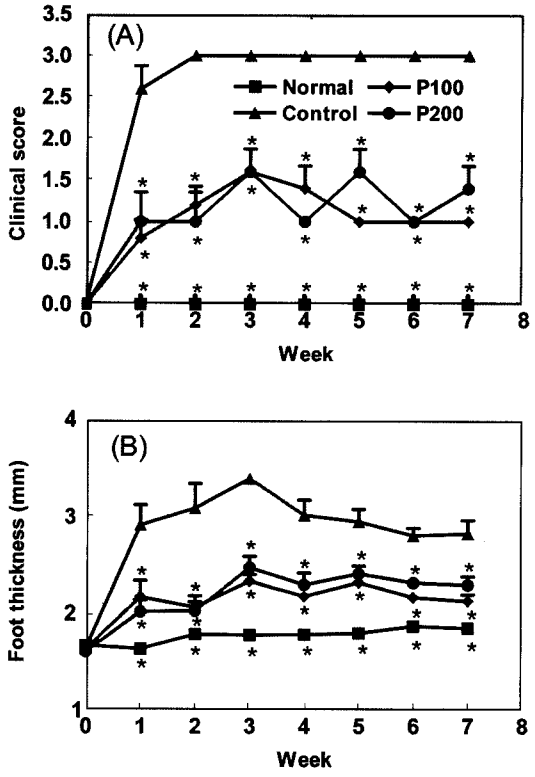

Fig. 1 Progression of established CIA in DBA/1J mice. (A) Clinical score, (B) Foot thickness. Normal, non-immunized normal mice; control, CIA mice not treated with sample; P100, CIA mice treated with potato extract (100 mg/kg); P200, CIA mice treated with potato extract (200 mg/kg). Results are expressed as mean± SEM (n=7). *Significantly different from control, $p<0.05$.

the Fc-portion of the IgG from various species. RF ELISA provides a rapid, sensitive, and semi-quantitative measurement of total IgGs RF to study the development and progression of RF-associated diseases in a mouse model (35). We examined the inhibitory effect of potato extract on the production of RF in the serum of mice with CIA (Table 2). Oral administration of potato extract (100 and 200 mg/kg) reduced the formation of RF in the CIA mouse serum. RF titer, particularly that of IgG RF correlates with the intensity of synovitis. RF can be found in serum samples from a variety of autoimmune diseases including rheumatoid arthritis, systemic lupus erythematous, Sjogren's syndrome, mixed connective tissue disease, mixed essential cryoglobulinemia, scleroderma, subacute

Table 1. Levels of AST, ALT, and creatinine in the blood of DBA/1J mice

Group[1)]	AST (U/mg protein)	ALT (U/mg protein)	Creatinine (μg/mg protein)
Normal	212.71±32.992[b2)]	369.55±26.768[a]	3.6935±0.2258[ns]
Control	317.96±28.952[a]	397.88±26.828[a]	4.0013±0.9728[ns]
P100	248.17±18.443[ab]	236.25±20.757[b]	3.1253±0.1455[ns]
P200	196.83±18.687[b]	318.44±26.034[ab]	2.8384±0.0356[ns]

[1)]Normal, non-immunized normal mice; control, CIA mice not treated with sample; P100, CIA mice treated with potato extract (100 mg/kg); P200, CIA mice treated with potato extract (200 mg/kg). [2)]Groups with different letters in the same column are significantly different from each other, $p<0.05$; [ns]: not significant.

bacterial endocarditis, leprosy, pulmonary fibrosis, and pulmonary silicosis (34). Heterologous type II collagen is widely used as an immunogen for the CIA model. In CIA-susceptible mice, the serum levels of antibodies to the type II collagen used for immunization are very high. These antibodies are also highly cross-reactive to various species of type II collagen. Although type I collagen shares more than 80% of the same amino acid sequences with type II collagen, it is not capable of inducing autoimmune-mediated arthritis and the antibody epitopes on collagen. In the present study, all mice sensitized to collagen produced these antibodies (Table 2). Serum anti-type II collagen IgG was barely detected in the normal group. In the groups treated with potato extract, the anti-CII antibody level was significantly suppressed. This result is consistent with the reduced RF observed in this group.

To obtain insight into the potential mechanisms involved in the amelioration of CIA by potato extract, the secretion of different cytokines (TNF-α, IL-1β, and IL-6) was investigated (Table 2). As expected, serum levels of all the cytokines analyzed were higher in arthritic mice than in nonarthritic mice. Treatment with potato extract for 49 days resulted in a decrease in the levels of IL-1β and IL-6 assayed. The excessive production of proinflammatory cytokines like TNF-α, IL-1β, and IL-6, has been implicated as a major factor in the pathogenesis of the disease (36). Moreover, both TNF-α and IL-1β have been detected in the joints of rheumatoid arthritis patients (37). TNF-α and IL-1β seem to function synergistically to induce the synthesis and secretion of IL-6 as effectors. It has been well demonstrated that TNF-α, IL-1β, and IL-6 are highly expressed at sites of diseases in collagen-induced arthritis (38). In the course of the exacerbation phase of rheumatoid arthritis, inflammatory cells invade the synovial cavity and produce inflammatory cytokines (1). These cytokines accelerate pannus formation and finally cause cartilage damage and bone destruction. Thus, the regulation of these cytokines may be important in the pathogenesis and therapy of rheumatoid arthritis (39). Our study suggests that potato extract may be useful in prevention of rheumatoid arthritis caused by chronic inflammation. The inhibitory effect of potato extract seen in the CIA mice could be due to a halting of disease progression by the inhibition of cellular infiltration into joints. The treatments could also improve clinical scores by inhibiting the production of cytokines and other inflammatory mediators from leukocytes already resident in the joint. Meanwhile, the overall effect of potato extract on the CIA model did not show dose-dependence.

Decomposition of peroxidized lipid yields a wide variety of end-products, including malondialdehyde (MDA). Significantly elevated levels of MDA in the serum of CIA mice were observed in the present study (Table 3) and similar results have been reported by other workers (40-42). Treatment with potato extract significantly reduced serum MDA levels when compared to the CIA group, suggesting that potato extract may have an antioxidant activity in CIA mice. Lipoproteins are macromolecules of lipid and protein that transport lipids (including cholesterol and triglycerides) through the vascular and extravascular body fluids. They are involved in a diversity of processes such as immune reactions, coagulation, and tissue repair. Increases in HDL-cholesterol and reduction of TG, total-cholesterol, and LDL-cholesterol are considered to help prevent coronary artery disease. Table 3 also shows the effects of potato extract on serum lipid profiles in mice. Daily intake of potato extract (100 and 200 mg/kg) for 7 weeks resulted in significantly lower serum LDL-cholesterol than that seen in the CIA control group ($p<0.05$) was observed. From the above consideration, it seems that potato extract may take part in the improvement of lipid metabolism.

There is evidence indicating that a low antioxidant status is associated with a higher risk of developing RA (43). Free radicals are capable of damaging a variety of biologically important compounds. The biological effects

Table 2. Levels of parameters related to RA in the blood of DBA/1J mice

Group[1)]	Reumatoid factor (AU/mg)	Anti-collagen antibody (U/mg)	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Normal	0.3675±0.0118[c2)]	0.5207±0.0079[b]	16.207±0.3448[ns]	12.143±0.8248[b]	13.686±5.1432[b]
Control	0.7445±0.0026[a]	5.8379±1.3111[a]	17.506±0.4062[ns]	16.905±0.2381[a]	41.333±9.0454[a]
P100	0.5947±0.0589[b]	2.3443±0.7197[b]	16.092±0.1149[ns]	4.7619±1.3257[c]	11.529±2.780[b]
P200	0.5597±0.0201[b]	1.1168±0.1022[b]	15.839±1.7853[ns]	3.8095±1.3257[c]	14.863±1.7428[b]

[1)]Normal, non-immunized normal mice; control, CIA mice not treated with sample; P100, CIA mice treated with potato extract (100 mg/kg); P200, CIA mice treated with potato extract (200 mg/kg). [2)]Groups with different letters in the same column are significantly different from each other, $p<0.05$; [ns]: not significant.

Table 3. Lipid profiles in the blood of DBA/1J mice

Group[1)]	MDA (nmole/mg)	TG (mg/dL)	Total cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	HDL-cholesterol (mg/dL)
Normal	1.6513±0.1385[c2)]	136.01±13.037[a]	340.00±7.7739[a]	214.95±6.0237[b]	97.850±2.3976[b]
Control	4.3596±0.2644[a]	181.18±7.7349[a]	374.55±17.794[a]	246.85±7.4974[a]	104.86±4.9165[ab]
P100	2.3317±0.354[b]	158.23±14.651[a]	360.91±8.4428[a]	228.61±6.2364[b]	100.65±3.2273[b]
P200	2.1650±0.0973[b]	148.03±5.9644[a]	363.64±2.2727[a]	223.24±7.0882[b]	113.55±6.4242[a]

[1)]Normal, non-immunized normal mice; control, CIA mice not treated with sample; P100, CIA mice treated with potato extract (100 mg/kg); P200, CIA mice treated with potato extract (200 mg/kg). [2)]Groups with different letters in the same column are significantly different from each other, $p<0.05$.

Table 4. Antioxidant enzyme activity in the spleen of DBA/1J mice[1)]

Group[2)]	SOD (U/mg protein)	GPx (U/mg protein)	GR (U/mg protein)
Normal	0.1644±0.0365[a3)]	0.0848±0.0045[a]	0.8372±0.0372[a]
Control	0.0792±0.0045[b]	0.0485±0.0007[b]	0.4897±0.0053[c]
P100	0.1176±0.0042[b]	0.0547±0.0021[b]	0.5819±0.0175[b]
P200	0.0953±0.0108[b]	0.0797±0.0070[a]	0.7785±0.0571[a]

[1)]SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, Glutathione reductase.
[2)]Normal, non-immunized normal mice; control, CIA mice not treated with sample; P100, CIA mice treated with potato extract (100 mg/kg); P200, CIA mice treated with potato extract (200 mg/kg).
[3)]Groups with different letters in the same column are significantly different from each other, $p<0.05$.

of free radicals are controlled *in vivo* by a wide spectrum of defence mechanisms such as SOD, GPx, and GR. In the present study, significantly low antioxidant enzyme activities were observed in the spleen of CIA mice when compared with normal mice (Table 4). These results suggest that oxidative stress on the spleen's immune system may play an important role in RA. Localized tissue damage initiates inflammatory responses. Stimulated neutrophils, a source of superoxide anion radicals, may also contribute to spleen damage in mice with CIA. The inhibition of enzyme activity leads to inability of the spleen to convert oxidized oxidized glutathione (GSSG) to reduce GSH. The depression of GR activity may also reflect perturbation in the NADPH metabolism in CII-treated mice. This is a direct reflection of the toxicity of CIA and the inability of the spleen to maintain glutathione homeostasis. Treatment with potato extract significantly increased activities of GPx and GR in the spleens of mice with CIA. This suggests that potato extract may help the spleen to maintain the antioxidant system and to develop resistance to CIA-induced oxidative injury.

In conclusion, results of the present study clearly demonstrate that potato extract exerts anti-inflammatory and anti-oxidant actions in the CIA model and is thus found to be effective against chronic inflammatory conditions. The biological mechanism of potato extract in inflammatory diseases has not been fully elucidated, but it is hoped that the present study will contribute to the development of clinical treatments for chronic inflammatory diseases like rheumatoid arthritis.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by Korean Government (MOEHRD)(KRF-2004-206-E00013).

References

1. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu. Rev. Immunol. 14: 397-440 (1996)
2. Arend WP. Physiology of cytokine pathways in rheumatoid arthritis. Arthritis Rheum. 45: 101-106 (2001)
3. Choi EM. Effects of ethanol extract of *Ligularia fischeri* leaves on Freund's complete adjuvant-induced model of chronic arthritis in mice. Food Sci. Biotechnol. 15: 277-282 (2006)
4. Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J. Appl. Toxicol. 21: 69-73 (2001)
5. Aeseth J, Haugen M, Forre O. Rheumatoid arthritis and metal compounds - Perspectives on the role of oxygen radical detoxification. Analyst 123: 3-6 (1998)
6. Kalavacherla US, Ishaq M, Rao URK, Sachindranath A, Hepsiba T. Malondialdehyde as a sensitive marker of inflammation in patients with rheumatoid arthritis. J. Assoc. Physicians India 42: 775-776 (1994)
7. Czernichow S, Hercberg S. Interventional studies concerning the role of antioxidant vitamins in cardiovascular diseases: a review. J. Nutr. Health Aging 5: 188-195 (2001)
8. Wooley PH, Dutcher J, Widmer MB, Gillis S. Influence of a recombinant human soluble tumor necrosis factor receptor FC fusion protein on type II collagen-induced arthritis in mice. J. Immunol. 151: 6602-6607 (1993)
9. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 283: 666-668 (1980)
10. Takagishi K, Yamamoto M, Maeda K, Nishimura A, Yonemoto K, Hotokebuchi T, Kaibara N. Effect of deoxyspergualin on collagen arthritis in mice. Clin. Immunol. Immunop. 56: 81-87 (1990)
11. Singh G. Recent considerations in nonsteroidal anti-inflammatory drug gastropathy. The Am. J. Med. 105: 31S-38S (1998)
12. Friedman M, McDonald GM. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. Crit. Rev. Plant Sci. 16: 55-132 (1997)
13. Friedman M, Fitch TE, Levin CE, Yokoyama WH. Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. J. Food Sci. 65: 897-900 (2000)
14. Gubarev MI, Enioutina EY, Taylor JL, Visic DM, Daynes RA. Plant derived glycoalkaloids protect mice against lethal infection by *Salmonella typhimurium*. Phytother. Res. 12: 79-88 (1998)
15. Cham BE. Solasodine glycosides as anticancer agents: pre-clinical and clinical studies. Asia Pac. J. Pharmacol. 9: 113-118 (1994)
16. McGehee DS, Krasowski MD, Fung DL, Wilson B, Gronert GA, Moss JM. Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. Anesthesiology 93: 510-519 (2000)
17. Heal KG, Sheikh NA, Hollingdale MR, Morrow WJ, Taylor-Robinson MW. Potentiation by a novel alkaloid glycoside adjuvant of a protective cytotoxic cell immune response specific for preery-throcytic malaria candidate antigen. Vaccine 19: 4153-4161 (2001)
18. Friedman M, Henika PR, Mackey BE. Effect of feeding solanidine, solasodine, and tomatidine to non-pregnant and pregnant mice. Food Chem. Toxicol. 41: 61-71 (2003)
19. Choi EM, Koo SJ. Anti-nociceptive and anti-inflammatory effects of the ethanolic extract of potato (*Solanum tuberlosum*). Food Agric. Immunol. 16: 29-39 (2005)
20. Choi EM, Lee KH, Koo SJ. Effects of ethanol wxtract of potato (*Solanum tuberlosum*) on Freund's complete adjuvant-induced model of chronic arthritis in mice. Food Sci. Biotechnol. 14: 228-232 (2005)
21. Guide for the Care and Use of Laboratory Animals. Grossblatt N (ed). Institute of Laboratory Animal Resources, National Academy Press, Washington, DC, USA (1996)
22. Hong H, Johnson P. Antioxidant enzyme activities and lipid peroxidation levels in exercised and hypertensive rat tissues. Int. J. Biochem. Cell B 27: 923-931 (1995)

23. Williams RO, Feldmann M, Maini RN. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. P. Natl. Acad. Sci. USA 89: 9784-9788 (1992)
24. Placer ZA, Cushmann LL, Johnson BC. Estimation of products of lipid peroxidation (as malondialdehyde) in biochemical systems. Anal. Biochem. 16: 359-364 (1966)
25. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47: 469-474 (1974)
26. Flohe L, Gunzler WA. Glutathione peroxidase. Method Enzymol. 105: 115-121 (1984)
27. Carlberg I, Mannervik B. Glutathione reductase. Method Enzymol. 113: 484-499 (1985)
28. Luross JA, Williams NA. The genetic and immunopathological processes underlying collagen-induced arthritis. Immunology 103: 407-416 (2001)
29. Whitehouse MW, Orr KT, Beck FWJ, Pearson CM. Freund's adjuvants: relationship of arthritogenicity and adjuvanticity in rats to vehicle composition. Immunology 27: 311-315 (1974)
30. Latha RM, Geetha T, Varalakshmi P. Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. Gen. Pharmacol. 31: 601-606 (1998)
31. Wang Y, Fang Y, Huang W, Zhou X, Wang M, Zhong B, Peng D. Effect of sinomenine on cytokine expression of macrophages and synoviocytes in adjuvant arthritis rats. J. Ethnopharmacol. 98: 37-43 (2005)
32. Whitehouse MW, Beck FJ. Impaired drug metabolism in rats with adjuvant-induced arthritis: a brief review. Drug Metab. Dispos. 1: 251-255 (1973)
33. Hung DY, Siebert GA, Chang P, Whitehouse MW, Fletcher L, Crawford DHG, Roberts MS. Hepatic pharmacokinetics of propranolol in rats with adjuvant-induced systemic inflammation Am. J. Physiol.-Gastr. L. 290: G343-G351 (2006)
34. Mahajan A, Sharma R, Khajuria R, Bardi GH, Kapoor B, Gupta V. Rheumatoid arthritis: new developments in biologic therapy. J. Indian Med. Assoc. 104: 327-330 (2006)
35. Tighe H, Chen PP, Tucker R, Kipps TJ, Roudier J, Jirik FR, Carson DA. Function of B cells expressing a human immunoglobulin M rheumatoid factor autoantibody in transgenic mice. J. Exp. Med. 177: 109-118 (1993)
36. Cannella AC, O'Dell JR. Early rheumatoid arthritis: pitfalls in diagnosis and review of recent clinical trials. Drugs 66: 1319-1337 (2006)
37. Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cytokines in cartilage destruction and repair. Brit. J. Rheumatol. 31: 653-661 (1992)
38. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheum. 38: 151-160 (1995)
39. Marinova-Mutafchieva L, Williams RO, Mason LJ, Mauri C, Feldmann M, Maini RN. Dynamics of proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis (CIA). Clin. Exp. Immunol. 107: 507-512 (1997)
40. Biemond A, Swaak AJG, Koster JF. Protective factors against oxygen free radicals and hydrogen peroxide in rheumatoid arthritis synovial fluid. Arthritis Rheum. 27: 760-765 (1984)
41. Özgünes H, Gürer H, Tuncer S. Correlation between plasma malondialdehyde and ceruloplasmin activity values in rheumatoid arthritis. Clin. Biochem. 28: 193-194 (1995)
42. Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin. Biochem. 30: 351-355 (1997)
43. Knekt P, Heliovaara M, Aho K, Alfthan G, Marniemi J, Aromaa A. Serum selenium, serum alpha-tocopherol, and the risk of rheumatoid arthritis. Epidemiology 11: 402-405 (2000)