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Effects of Ethanol Extract of Potato (*Solanum tuberlosum*) on Freund's Complete Adjuvant-induced Model of Chronic Arthritis in Mice

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Abstract The effect of a potato ethanol extract was investigated on Freund's complete adjuvant (FCA)-induced arthritis. The oral administration of the potato extract, at doses of 100 and 200 mg/kg once a day for 21 days, significantly reduced hindpaw swelling and the production of inflammatory cytokines (TNF-α, IL-1β and IL-6). Treatment with the potato extract also decreased the plasma levels of creatinine, triglyceride and LDL-cholesterol, and the activities of AST and ALT compared with those of a control group. These results suggest that the potato extract might be beneficial in the treatment of chronic inflammatory disorders.

Keywords: *Solanum tuberosum*, inflammation, Freund's complete adjuvant, arthritis

Introduction

Inflammatory diseases, including different types of rheumatic disease, are very common throughout the world, and although rheumatism is one of the oldest known diseases of mankind, affecting the majority of the population, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and the reappearance of symptoms after discontinuation. Therefore, the screening and development of agents for their anti-inflammatory activities are still in progress, with much hope being ascribed to indigenous medicinal plants (1).

Potato (*Solanum tuberosum* L.) is one of the main widely consumed vegetables. In Korea, the raw juice of peeled potato has been recommended as a remedy for various ailments, and has been used for treating various inflammatory diseases, such as articular rheumatism and rheumatic pains (2). The health benefits of potato consumption seem to be related, at least partly, to the antioxidant phenolic metabolites content. Moreover, potato is known to contain alkaloids. Steroidal glycoalkaloids are naturally occurring secondary plant metabolites formed in a number of foods, including potatoes, tomatoes and eggplants (3, 4). Although they are reported to be potentially toxic, glycoalkaloids, and their hydrolysis products, without the carbohydrate side chain (aglycons) also have beneficial effects. These include lowering of cholesterol (5), protection against infection by *Salmonella typimurium* (6) as well as cancer (7), and potentiation of general anesthetics, which act by inhibiting cholinesterase (8), and of a malaria vaccine (9).

Arthritis-related joint problems include oedema, inflammation and damage to the cartilage and surrounding structures. Particularly, these chronic insults affect the sensory nerves, innervating the arthritic joint (10). A Freund complete adjuvant (FCA) injection results in a localized monoarthritis, enabling study of arthritic lesions without the complicating factors of poor animal mobility, altered weight gain and systemic disease. Arthritis-related syndrome is characterized by the development of chronic joint inflammation, with cell proliferation, synovium enlargement, pannus formation and destruction of joint cartilage (11). The mouse model of adjuvant-induced arthritis has been used for many years for evaluation of anti-arthritic/anti-inflammatory agents, and has been well characterized (12), and has also been useful in the development of newer therapeutic agents. In the present study, we investigated the effects of a 70% ethanolic extract of potato, orally administration daily for 21 days, on the inflammatory reactions in a mouse experimental model of arthritis. The effects of the extract on the biochemical parameters were also studied.

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Materials and Methods

Animal Male ICR mice (6 weeks old) were purchased from Jungang Lab Animal Inc (Korea). These animals were maintained under constant temperature (24±2°C), with a 12h light-dark cycle, relative humidity 40-70% and allowed food and water *ad libitum*. Handling and sacrificing of the animals were conducted in full accordance with national and international laws and policies and the National Institute of Health's Guide for the Care and Use of Laboratory Animals (13).

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

Induction of arthritis by Freund complete adjuvant

(FCA) Mice were randomly divided into five groups (n =7), these being a normal untreated control group, control group injected with FCA and an orally administered vehicle, and treated groups injected with FCA and orally administered indomethacin (10 mg/kg) and potato extract (100 and 200 mg/kg) daily for 21 days. The body weights were measured before injection of the adjuvant (day 0). Each mouse was then injected with 0.1 ml of Freund's complete adjuvant (1mg/ml in mineral oil; sigma, St. Louis, MO) in the right posterior plantar region (14). Test samples (potato extract and indomethacin) were prepared by suspending them in 0.5% sodium carboxymethyl cellulose (CMC-Na) solution, immediately before the start of the experiments. The pad thicknesses of the hind paws were measured with a Dial Thickness Gauge (Mitutoyo, Japan) before and day 21 after the FCA injection, and the difference in the thicknesses calculated. The degree of foot-pad swelling was expressed as an increase in foot-pad thickness (mm). On day 21, after an adjuvant injection, the body weights of the mice fasted overnight were obtained from the groups of mice before sacrifice. Blood was then collected from the caudal vena cava and placed in heparinized tubes. Plasma was prepared by centrifugation at 4°C and 2000×g for 15min, and stored at -70°C. The liver, spleen and kidneys were removed and weighed.

Measurement of cytokines in plasma The cytokine levels in the plasma were determined at room temperature using ELISA kits for murine TNF-α, IL-1β and IL-6 (R&D Systems, USA), according to the manufacturer's instructions.

Assay for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine in plasma The AST and ALT activities and creatinine level in the plasma were assayed using commercial kits (YD Diagnostics. Korea), according to the manufacturer's recommended procedures.

Determination of triglyceride and cholesterol levels The concentrations of TG, total cholesterol and HDL-cholesterol in the plasma were determined enzymatically using commercial available kit reagents (Boehringer, Mannheim, Germany). The LDL-cholesterol was calculated by Friedewald formula: LDL cholesterol = total cholesterol - HDL cholesterol - triglyceride / 5 (15).

Statistical analysis The results are expressed as the means ± SEM (n=5). Statistical significance was determined by an analysis of variance and subsequent Duncan's multiple range test ($P<0.05$). The analysis was performed using the SAS statistical software.

Results and Discussion

Body weight gain, organ weight and biological parameters in plasma of mice injected with FCA For the assessment of chronic inflammation, arthritis was induced by a FCA injection, with the potato extract administered orally at doses of either 100 or 200 mg/kg daily for 21 days. The body weight gains of the mice are given in Table 1. Over the study period, the body weight naïve control (normal) mice steadily increased. The body weights of the adjuvant-injected mice also increased, but less than that of the naïve control mice. The weights of liver and spleen of the FCA-injected mice were significantly increased compared with the normal control group. However, no difference was observed in the weights of the organs belonging either to the normal control or to potato extract-treated mice groups. The hepatoprotective effect was correlated with the decrease in both the AST and ALT values. As shown in Table 2, the administration of potato extract caused no increases in the AST and ALT activities, which were used to assess the hepatocyte integrity. The plasma creatinine level, used to assess kidney function, was slightly increased in the FCA-treated control group, but significantly decreased in the potato extract (100 mg/kg)-treated group (Table 2). These results indicated no liver and kidney failure in the potato extract treated mice. To corroborate the apparent lack of toxicity in both the kidney and liver, as suggested above, by an analysis of blood parameters, a

Table 2. Effects of potato extract on the AST and ALT activities and creatinine level in the plasma of mice

Group	AST (Unit/mg protein)	ALT (Unit/mg protein)	Creatinine (mg/dl)
Normal	349.59±11.30^{a}	83.34±6.83^{a}	0.48±0.04^{ab}
Control	404.80±14.60^{a}	107.43±3.41^{a}	0.57±0.05^{a}
Indo.	349.22±8.69^{a}	85.35±5.00^{a}	0.57±0.03^{a}
P100	403.79±8.86^{a}	115.38±9.48^{a}	0.28±0.08^{c}
P200	361.39±18.03^{a}	113.62±3.91^{a}	0.31±0.09^{bc}

Results are the means±SEM (n=7). Groups with different letters are significantly different from each other by analysis of variance ($P<0.05$). Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo: arthritic animals treated with indomethacin (10 mg/kg), P100: arthritic animals treated with potato extract (100 mg/kg), P200: arthritic animals treated with potato extract (200 mg/kg)

Table 1. Body weight gain and organ weight of mice

Group	Weight (g)			
	Body Weight	Liver	Kidney	Spleen
Normal	4.80±1.49^{a}	1.15±0.08^{c}	0.37±0.02^{bc}	0.12±0.0^{b}
Control	1.20±1.01^{a}	1.8±0.08^{a}	0.41±0.02^{ab}	0.24±0.01^{a}
Indo.	0.50±0.31^{a}	1.65±0.14^{b}	0.44±0.02^{a}	0.16±0.06^{b}
P100	1.50±1.52^{a}	1.08±0.17^{c}	0.33±0.02^{c}	0.13±0.06^{b}
P200	1.20±1.01^{a}	1.09±0.14^{c}	0.34±0.02^{c}	0.13±0.05^{b}

Results are the means±SEM (n=7). Groups with different letters are significantly different from each other by an analysis of variance ($P<0.05$). Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo: arthritic animals treated with indomethacin (10 mg/kg), P100: arthritic animals treated with potato extract (100 mg/kg), P200: arthritic animals treated with potato extract (200 mg/kg)

histopathological study of these organs was also carried out. Histological examinations of the liver and kidney, used as target organs, showed no marked differences between the normal control and potato extract treated groups, and no evident alterations were detected (results not shown). According to our results, it can be concluded from the biochemical results obtained that the consumption of potato extract has no negative effect on the mouse liver hepatocyte integrity or function.

Effects of potato extract on the FCA-induced paw edema A FCA injection resulted in a significant increase in paw thickness, with a doubling of paw edema compared with the normal control group after the 21 days of monitoring (Fig. 1). Histological sections of the FCA-treated paws taken on day 22 showed the presence of chronic inflammation, with neutrophilic and monocytic infiltration of the sub-synovial tissues (data not shown). By the 22nd day, deformities, especially in the hind paws, were evident in the animals on visual inspection. The results in Fig. 1 show that potato extract caused significant ($P<0.05$) inhibition of paw edema compared to the normal control group, with similar inhibitory potency to that of indomethacin (10 mg/kg). Gauldie et al. (10) reported that seven days of treatment with either indomethacin (NSAID) or prednisolone (steroid) caused a significant decrease in joint inflammation and associated hyperalgesia. In the case of natural products, the anti-inflammatory effects of various edible plants have been reported (1, 16). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, although relatively little knowledge is available on their mode of action.

Effects of potato extract on the TNF-α, IL-1β and IL-6 levels in plasma of mice injected with FCA The production of cytokines by leukocytes and other body cells facilitates intercellular signalling during the activation of innate and specific immunity. These potent effects or molecules regulate a wide array of physiological and pathophysiological processes, including the initiation and coordination of immune, acute phase and inflammatory responses, and as a consequence play important roles in human health and disease (17). Under resting conditions, the serum cytokines are kept at very low levels by an intricate network of co-stimulatory and feedback loops (18). However, the concentration and/or activity of many cytokines increases dramatically in response to stressful or pathophysiological conditions (19). Typically, there is a sequential release of several inflammation-associated cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6. The levels of pro-inflammatory cytokines are shown in Fig. 2. When compared with the baseline levels measured in normal mice, the FCA treatment induced pronounced changes in the plasma levels of pro-inflammatory cytokines. The TNF-α, IL-1β and IL-6 levels after 21 days of FCA injections into the

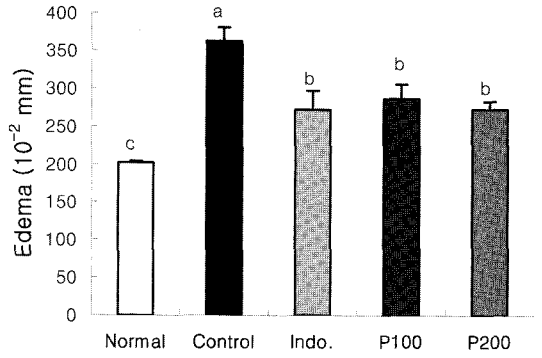

Fig. 1. Effects of potato extract on the FCA-induced paw edema. Results are expressed as the means±SEM (n=7). Bars without a common letter differ, $P<0.05$. Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo: arthritic animals treated with indomethacin (10 mg/kg), P100: arthritic animals treated with potato extract (100 mg/kg), P200: arthritic animals treated with potato extract (200 mg/kg).

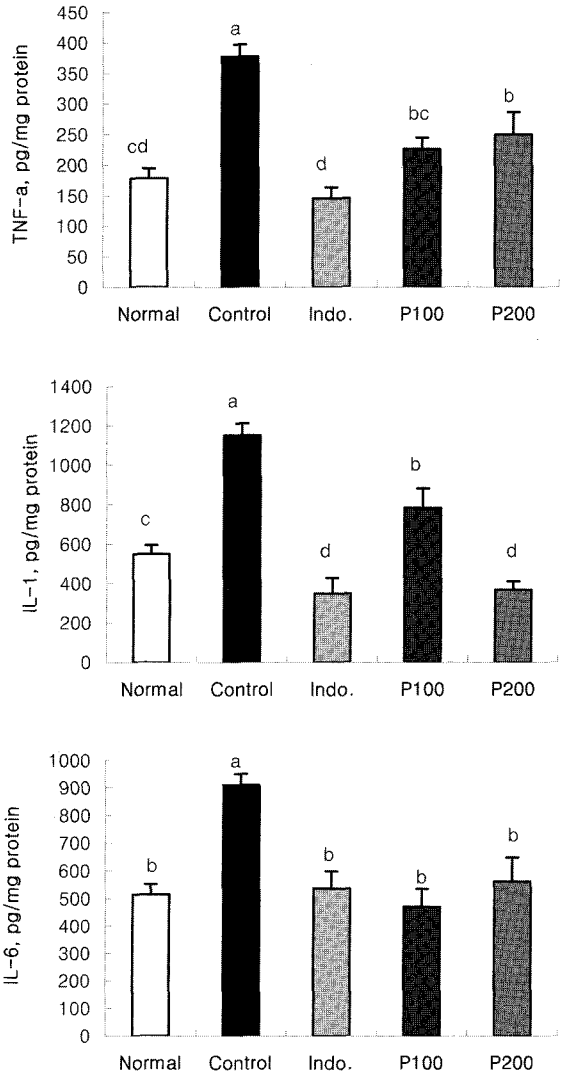

Fig. 2. Effects of potato extract on the TNF-α, IL-1β and IL-6 levels in plasma of mice injected with FCA. Results are expressed as the means±SEM (n=7). Bars without a common letter differ, $P<0.05$. Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo: arthritic animals treated with indomethacin (10 mg/kg), P100: arthritic animals treated with potato extract (100 mg/kg), P200: arthritic animals treated with potato extract (200 mg/kg)

Table 3. Concentrations of plasma lipids

Group	Plasma concentration (mg/100ml)			
	TG	Chol	LDL	HDL
Normal	145.79±11.17^{ab}	465.15±11.66^{a}	283.85±11.05^{b}	152.1±8.88^{ab}
Control	169.38±1.92^{a}	516.67±31.93^{a}	352.07±25.59^{a}	130.72±9.27^{b}
Indo.	117.98±17.26^{b}	463.64±25.81^{a}	291.09±17.96^{b}	148.95±3.81^{ab}
P100	125.98±5.07^{b}	486.36±23.62^{a}	301±17.70^{b}	160.16±8.61^{a}
P200	127.67±3.28^{b}	427.27±24.58^{a}	251.27±15.04^{b}	150.47±3.46^{ab}

Results are the means±SEM (n=7). Groups with different letters are significantly different from each other by analysis of variance ($P<0.05$). Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo: arthritic animals treated with indomethacin (10 mg/kg), P100: arthritic animals treated with potato extract (100 mg/kg), P200: arthritic animals treated with potato extract (200 mg/kg)

potato extract group were significantly ($P<0.05$) lower than those in the normal control group. A growing number of reports have demonstrated that indomethacin and other NSAIDs directly modulate cytokine production, both *in vivo* and *ex vivo* (20), altering the activities of certain transcription factors, including NF-κB (21).

Lipid profiles in plasma of mice injected with FCA Lipoproteins are macromolecules of lipids and proteins that transport lipids, including cholesterol and triglycerides, through the vascular and extravascular bodily fluids, and are involved in a diversity of processes, such as immune reactions, coagulation and tissue repair. An increase in the high-density lipoprotein, (HDL)-cholesterol, and reductions in triglyceride (TG), total-cholesterol and the low-density lipoprotein, (LDL)-cholesterol, are considered to constitute anti-coronary artery disease. Table 3 shows the results of the effects of potato extracts on the plasma lipid profiles in mice following its administration at 100 and 200 mg/kg body weight, for 3 weeks. The TGs and LDL-cholesterol contents were significantly lower in the potato extract-treated group than in the normal control group; the mice also presented normal total-cholesterol levels. Numerous epidemiological studies have associated HDL with an inverse risk for coronary artery disease. This protective effect of HDL may be due, in part, to an inhibition of the oxidative modification of LDL (22). A number of studies have confirmed that HDL attenuates the cytokine-induced expression of the adhesion molecules in cultured endothelial cells (23). Because TNF-α and IL-1β increase the expression of endothelial leukocyte adhesion molecules, due to activation of the transcription factor NF-kB, it is hypothesized that HDL inhibits the activation of NF-κB. HDL also suppresses the caspases required for apoptosis, which are activated by TNF-α, and appears to modulate the permeability of the endothelium to LDL. Large doses of HDL administered to rabbits decrease the appearance of LDL particles in the intima of the aorta (24). In the present study, a daily intake of potato extract (100 mg/kg body weight) for 3 weeks significantly increased HDL-cholesterol and decreased plasma LDL-cholesterol and TGs compared with the FCA-treated control group; effects which may be beneficial in the prevention of ischemic heart disease. From the above consideration, it seems that potato extract may take part in stimulating the performance capacity of lipid metabolism, with the associated characteristic physiological and biochemical properties.

In conclusion, the results of the present study clearly demonstrate that potato extract exerts potent anti-inflammatory actions in the FCA-induced chronic arthritis model; and thus, was found to be effective under chronic inflammatory conditions. Also, the above results indicated that the administration of 100 and 200 mg/kg of body weight/day of potato extract over 21 days showed no sign of toxicity, with most of the serum biochemical and hematological parameters remaining normal. In conclusion, potato extract might be proposed as a candidate for a novel treatment of chronic joint inflammation. Further detailed investigations are underway to determine the exact phytoconstituents responsible for the anti-inflammatory activity.

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