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Origianal Reseach

Anti-Fatigue Properties of Cultivated Wild Ginseng Distilled Extract and Its Active Component Panaxydol in Rats

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Key Words

ginseng, fatigue, force swimming test, lactate dehydrogenase, panaxydol

Abbreviations

BUN blood urea nitrogen
cWG cultivated wild ginseng
FST forced swimming test
i.p. intraperitoneally
LAC lactate acid
LDH lactate dehydrogenase
LPS lipopolysaccharide

Abstract

Objectives: Cultivated wild ginseng (cWG), called San-YangSanSam, has been used clinically in patients with chronic fatigue in Korea. Little is known about effects of the ginseng distilled (volatile) components produced during evaporizaiton. Recently, we first identified one major component from cWG distilled extract, panaxydol, by using mass spectrometry. However, functional properties of cWG distilled extract and panaxydol remains elusive. Therefore, the present study evaluated the effect of cWG distilled extract or panaxydol on exercise-induced fatigue in rats.

Methods: Fatigue was induced by forced swimming and the immobility time was analyzed in male Sprague-Dawley rats. The animals received intraperitoneally either vehicle, cWG distilled extract, or panaxydol 10 min prior to beginning of the forced swimming test (FST) once daily for 5 days. After the FST on day 5, we also analyzed fatigue-related biochemical levels including blood urea nitrogen (BUN), lactate acid (LAC), and lactate dehydrogenase (LDH) in serum and levels of glycogen in liver and soleus muscle.

Results: The forced swimming time in cWG distilled extract (0.6 mL/kg)-treated group was significantly longer than that of control group on day 4 and 5. Panaxydol (0.1 and 0.25 mg/kg)-treated groups showed significantly enhanced performance in the forced swimming, compared to control. In addition, a significant decrease in serum LDH level was found in panaxydol-treated group, while there were no alternations in levels of serum BUN and LAC and glycogen in liver or soleus muscle.

Conclusion: The present study demonstrated cWG distilled extract and its active component panaxydol have a function of anti-fatigue.

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1. Introduction

Fatigue is a feeling of extreme physical or mental tiredness and exhaustion as one of the common physiological reactions resulting from severe stress, hard physical or mental work. Fatigue may be associated with many disorders and mainly caused by the depletion of energy sources which include the accumulation of the end products of fatigue, the decrease in liver glycogen consumption [1]. In particular, physical fatigue is the transient inability of a muscle to maintain optimal physical performance and induced by intense physical exercise frequently leads to a deterioration in performance, causing a decrease in muscular power and endurance and in mental functions [2]. Ginseng is a well-known medicinal herb and has been traditionally used as a medicine for anti-tumor [3], anti-oxidant [4], anti-inflammatory [5] and hypoglycemic properties [6]. Ginseng has also been used to enhance physical strength, expectially in patients who suffered from severe fatigue [7, 8] and cancer-related fatigue [9]. Components of ginseng such as ginseng polysaccharide or small molecule oligopeptides isolated from the *Panax ginseng* have shown anti-fatigue activity and the effects on the physiological biomarkers for fatigue [10] and produced anti-fatigue effect by increasing the forced swimming time and enhancing lactic dehydrogenase (LDH) and glycogen levels in liver of mice [11].

As a type of ginseng, Korean wild ginsengs (*SanSam*, mountain ginseng) are naturally grown in deep mountains and quite rare and expensive in Korea. To mimic naturally grown ginsengs, ginsengs are often cultivated in deep mountains and classified into *SanYangSam* (ginseng cultivated in mountain) and *SanYangSanSam* (wild ginseng cultivated in mountain or cultivated wild ginseng, cWG), depending on the types of ginseng seeds. Crude or distilled extracts from cWG (called as *SanYangSanSam*) were reported to have anti-tumor or -cancer effect [12, 13]. Especially, the distilled extracts from cWG have been used clinically for injection into acupuncture points in Korea [13] and have also shown to reduce inflammation in lipopolysaccharide (LPS)-induced rat model [14] and oxidative stress in obese rats [15]. While the previous experimental studies have supported the effectiveness of cWG distilled extract, the active components of cWG distilled extract and their biological effects are largely unknown. Recently, by using liquid chromatography tandem mass spectrometry and quadrupole orthogonal acceleration time-of-flight mass spectrometry, we found that cWG distilled extract contained panaxydol as a major component and the level of panaxydol was about 30 times higher than those of ginseng cultivated in mountain (SanYangSam) [16]. As cWG distilled extract has long been used clinically in order to enhance physical energy in cancer patients suffering from fatigue, cWG distilled extract and its major component panaxydol may play a role in reducing fatigue.

Thus, the present study was designed to evaluate the anti-fatigue properties of cWG distilled extract and its active component panaxydol in rats by performing the forced swimming test and measuring fatigue-related biomarkers [17] such as blood urea nitrogen (BUN), lactic acid (LAC), LDH, and glycogen in liver and muscle of rats.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats were purchased from Daehan animal Co. (Seoul, South Korea) weighing 270-320 g and were housed in groups of 2-3 animals per cage at a room temperature of 22 ± 2°C with a 12 hr-light-dark cycle and received ad libitum food and water. All experimental procedures were conducted in accordance with National Institutes of Health guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee at Daegu Haany University and Daejeon University.

2.2. Experimental designs

Two separated experiments were conducted to test the hypothesis that anti-fatigue properties of cWG distilled extract and its active component panaxydol in rats by performing the forced swimming test and measuring fatigue-related biomarkers such as BUN, LAC, LDH, and glycogen in liver and muscle of rats. The first experiment was conducted to determine whether cWG distilled extract affects forced swimming time and glucogen levels in liver and soleus muscle. Thus, rats were divided into two experimental groups: saline-treated group (n = 6) and cWG distilled extract-treated group (n = 6). The second experiment was conducted to see whether panaxydol, an active component of cWG, alters forced swimming time, biochemical levels such as BUN, LAC, and LDH in serum and levels of glycogen in liver and soleus muscel. For this experiment, rats were divided into three experimental groups: vehicle (n = 5), panaxydol (0.1 mg/kg)-treated (n = 5), and panaxydol (0.25 mg/kg)-treated (n = 5) groups.

2.3. Drugs and chemicals

Cultivated wild ginseng (cWG, ChonBangNongSan Inc., Chungnam, Korea), about 8–10 years old, was used. The intact cWG was washed, dried, and crushed to super-fine powder (mean particle size, 7.5 μm) using a turbo mill. Distilled extract from cWC (*SanYangSanSam*, 20 ml/vial) was made in Korean Pharmacopuncture Research Institute (KPRI) as described previously [16] 16]. Voucher specimens (#CWG-2015-03-DE) have been deposited at the KPRI. In brief, the wild ginseng was washed with distilled water to remove debris and contaminants. Fourty grams of the dried wild ginseng were mixed with 250 mL distilled water in the round plask and heated at 80°C via a closed-loop extraction system (KyungSeo machinery Com., Incheon, Korea)(Fig. 1A). During 24 hr heating, the vapor was condensed by cooling in the closed-loop system and cWG distilled extract of about 200 mL was obtained. Panaxydol was purchased from Chengdu Biopurify Phytochemicals Ltd (Chengdu, China). Levels of BUN, LDH, LAC and glycogen were determined with the IDEXX VetTest Chemistry analyzer (IDEXX Laboratories, Westbrook, Maine, USA). Other

chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Panaxydol was dissolved in 100% ethanol and then diluted to saline before use. Vehicle, cWG distilled extract (0.6 mL/kg) or panaxydol (0.1 and 0.25 mg/kg, once a day) was intraperitoneally (i.p.) administered 10 min prior to the begining of forced swimming test (FST) for 5 days.

2.4. Forced swimming test

The force swimming test (FST) was carried out as described previously [1] . Briefly, rats were placed individually into a plastic container (30 x 30 x 80 cm) filled with water (25 ± 5°C) to a depth of 60 cm. A glass bar (10% of rat's body weight) was attached to the proximal part of the tail of rat. The total swimming time was recorded when the physical strength of rat was exhausted and it could not rise to the surface for more than 10 sec.

2.6. Measurement of tissue glycogen contents

Rats were euthanized under 4~5% isoflurane anesthesia after blood sample collections (2% isoflurane) to obtain liver and soleus mucle tissues. The glycogen levels in liver and soleus mucles were measured by using the method described previously [18] 18]. In brief, after sacrificing for blood collection, liver and soleus muscle were quickly dissected out, frozen in liquid nitrogen, and stored at -80°C until use. Each sample (20 mg per tissue) was boiled in 2.0 M HCl at 100°C for 1 hr and homogenized. After centrifugation, the samples were neutralized with 2.0 M NaOH and centrifuged again at 3000 rpm for 10 min. Level of glycogen was determined at 562 nm using a chemistry Analyzer VetTest 8008.

2.7. Statistical analysis

Data were carried out using SigmaStat 3 software (Systat Software, Inc, San Jose, CA, USA) and presented as the mean ± SEM (standard error of mean). Statistical analysis was analyzed by t-test, one-way or two-way repeated analysis of variance (ANOVA), followed by post hoc test using Tukey method. Statistical significance was considered at (*) $P < 0.05$ and (**) $P < 0.01$.

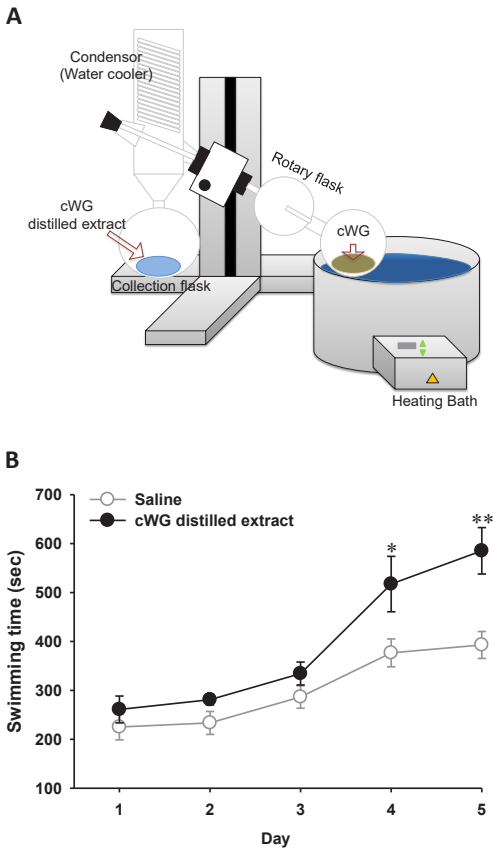


Figure 1 Effect of cultivated wild ginseng (cWG) distilled extract on the forced swimming time in rats.
A: A rotary evaporator used for collection of cWG distilled extract. B: rats were pretreated with saline or cWG distilled extract (0.6 mL/kg, i.p.) once daily 10 min prior to the forced swimming test for 5 days. Data were presented as mean ± SEM (n = 6 per group). *$P < 0.05$, **$P < 0.01$ vs. saline

3. Results

3.1. Effect of cWG distilled extract on the forced swimming test in rats.

The effect of cWG distilled extract on the forced swimming time of rats is shown in Fig.1. The forced swimming time of cWG distilled extract-treated group on day 4 and 5 was significantly longer than that of saline control group (Fig. 1B, repeated t-test; treatment $F(1,20) = 10.384$, $P = 0.023$; time $F(4,20) = 20.165$, $P < 0.001$; interaction $F(4,20) = 2.609$, P = 0.066).

3.2. Effects of cWG distilled extract on glycogen in liver or soleus muscle of rats.

To evaluate whether the effect of cWG distilled extract on forced swimming are associated with glycogen levels, glycogen levels were estimated in liver and soleus muscle of rats after the FST on day 5. As shown in Fig. 2A and 2B, cWG distilled extract did not affect the level of glycogen in liver (Fig. 2A: *t*-test, $F(1,10) = 0.056$, $P = 0.82$) or soleus muscle (Fig. 2B: *t*-test, $F(1,10) = 0.023$, $P = 0.88$) compared to saline control group.

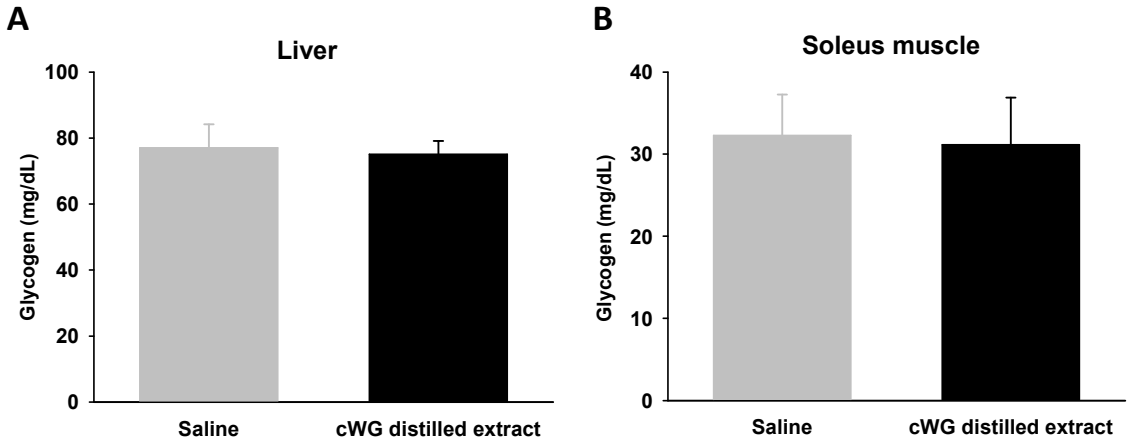


Figure 2 Effect of cultivated wild ginseng (cWG) distilled extract on serum level of glycogen in liver (A) and soleus muscle (B) after forced swimming test.

Rats were treated with saline or cWG distilled extract (0.6 mL/kg, i.p.) for 5 days. A: glycogen content in liver after the forced swimming. B: glycogen content in soleus muscle after the forced swimming. Data were presented as mean ± SEM (n = 6 per group).

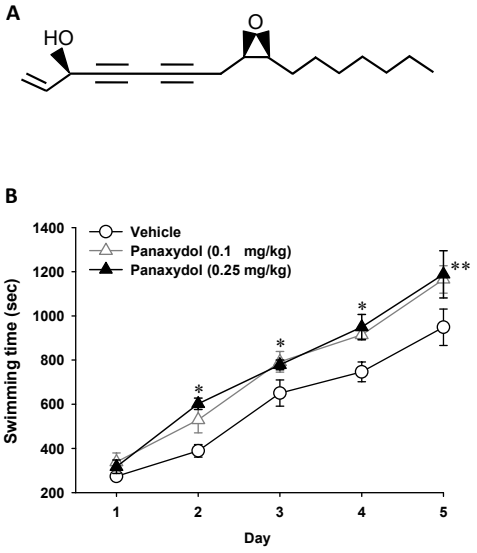


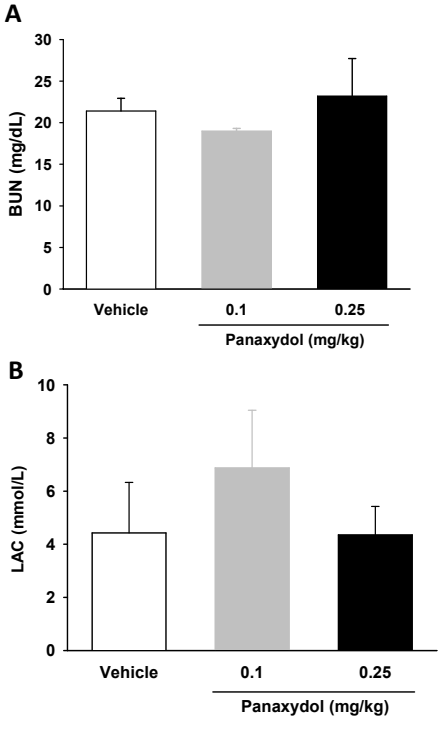
Figure 3 Effect of panaxydol on the forced swimming time in rats. A: chemical structure of panaxydol. B: effect of panaxydol on the forced swimming test. Animals were given vehicle or panaxydol (0.1 or 0.25 mg/kg, i.p.) once a day 10 min prior to the forced swimming test. Data were presented as mean ± SEM (n = 5 per group). *P < 0.05, **P < 0.01 vs. saline.

3.3. Effects of panaxydol on the forced swimming test in rats.

We tested the effect of panaxydol, a polyacetylenic compound found in *Panax ginseng*, on the FST in rats. As shown in Fig. 3B, panaxydol-treated groups (0.1 or 0.25 mg/kg) significantly increased forced swimming time compared to vehicle control group on day 4 and 5 (two-way, treatment F(2,32) = 21.997, P < 0.001; time F(4,32) = 120.756, P < 0.001; interaction F(8,32) = 1.531, P = 0.186).

3.4. Effects of panaxydol on serum biochemical parameters of rats.

Levels of fatigue-related serum biomarkers, BUN, LAC, and LDH were measured after last FST. Panaxydol had no effect on level of serum BUN (Fig. 4A; one-way, F(2,12) = 0.5842, P = 0.0573) or LAC (Fig. 4B; one-way, F(2,12) = 0.6488, P = 0.5401). However, LDH level was increased by forced swimming, which was significantly attenuated by treatment with panaxydol at dose of 0.1 mg/kg for 5 days (Fig. 4C; one-way, F(2,10) = 10.65, P = 0.003, P < 0.01 vs. vehicle).



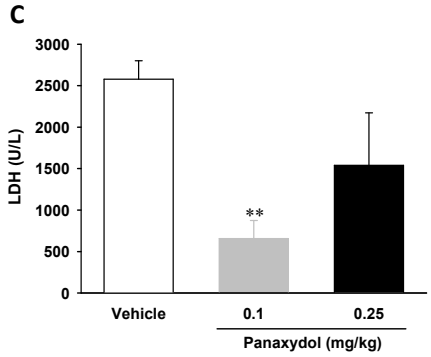


Figure 4 Effect of panaxydol on serum level of fatigue-related biomarkers after forced swimming test.
Rats were pretreated with vehicle or panaxydol (0.1 or 0.25 mg/kg, i.p.) 10 min prior to the forced swimming test. A: BUN, B: LAC, C: LDH after the forced swimming. Data were presented as mean ± SEM (n = 5 per group). **P < 0.01 vs. vehicle.

3.5. Effects of panaxydol on glycogen content in liver and soleus muscle tissue after forced swimming.

We also measured the level of glycogen in liver and soleus muscle of rats after the FST on day 5. As shown in Fig. 5A and 5B, panaxydol had no significant effect on level of glycogen in liver (Fig. 5A: one-way, $F(2,12) = 0.256$, $P = 0.78$) or soleus muscle (Fig. 5B: one-way, $F(2,12) = 0.715$, $P = 0.51$) compared to vehicle control group.

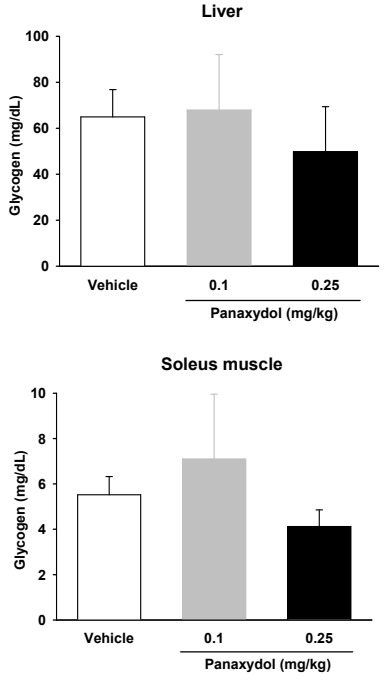


Figure 5 Effect of panaxydol on glycogen content in liver and soleus muscle of rats.
A: glycogen content in liver after the forced swimming. B: glycogen content in soleus muscle after the forced swimming. Data were presented as mean ± SEM (n = 5 per group).

4. Discussion

Ginseng has various pharmaceutical properties such as anti-tumor [3], anti-oxidant [4] and anti-inflammatory activities [5] and recovery of impaired memory [19]. Panaxidol, a ployacetylene compound isolated from ginseong, has shown a potential anti-cancer agent [20], a protective effct on neurodegeneration in cortical neurons [21], and a mimic the effect of neurve growth factor in PC 12 cells [22] . Several studies have also proved the effectiveness of ginseng or main active components of ginseng, such as ginsenosides Rb1 and small molecule oligopeptieds on exercise fatigue or cancer-related fatigue [11, 23, 24]. Most previous studies have utilized extract ginseng aqueous crude extracts collected through the extraction/condensation steps: ginseng roots are soaked in water or methanol, filtered and condensed by removing excessive water under vaccums. On the other hand, little is known about effects of ginseng distilled (volatile) components produced during evaporizaiton.

The present study shows for the first time, to our knowledge, that distilled extract from ginseng and its active component panaxidol had an anti-fatigue activity in the rat FST model.

Fatigue is one of the most common physiological reactions occured from exercise, depression, aging, cancer, multiple sclerosis, and Parkinson's disease. The FST is the most valid models for evaluation of anti-fatigue activity of a wide variety of food or plant compounds [2, 25-27] 25-27]. Consistent with a previous study [28] 28], our results revealed that cWG distilled extract increased the forced swimming time compared to saline control group. In addition, panaxidol, a major component of cWG, also increased the forced swimming time. These result indicates that anti-fatigue effect of ginseng may be associated with the activity of panaxidol in the forced swimming-inducd fatigue.

Exercise-induced fatigue such as forced swimming can be evaluated with biochemical indicatiors, including BUN, LAC and LDH levels in blood. Thus, we measured the level of serum BUN, LAC, and LDH in the rats given forced swimming and panaxydol treatement. Blood urea nitrogen is a metabolic product of proteins and amino acids, used an important indicator for evaluating exercise endurance and fatigue status [29] 29]. Lactate acid is considered a major indicator of muscle fatigue. Intense exercise leads to accummulation of lactate resulting in lowering pH of blood and muscle and consequent generation of fatigue [30] 30]. Lactate dehydrogenase is an index of muscle damage and catalyzes the interconversion of pyruvate and NADH+ to L-lactate in muscle cells. In our present study, repeated treatments with panaxydol at dose of 0.1 mg/kg significantly attenuated increased level of serum LDH (major enzyme for lactate production) in forced swimming rats while the levels of serum BUN or LAC were not changed. It may suggest that enhanced performance in forced swimming is associated with LDH. However, it is required to explore how panaxydol can reduce activity of LDH and muscle damages. Glycogen, which is main storage form of glucose, in liver or muscle is also an index of fatigue [31]

31]. Glycogen in liver complements the consumption of blood glucose to maintain blood glucose in the physiologic range and fatigue occurs when hepatic glycogen is mostly consumed [32, 33]. In our present study, glycogen levels in liver or soleus muscle of cWG- or panaxydol-treated rats were unaltered. Taken together, these findings suggest that panaxydol may improve physical fatigue via regulation of serum LDH level in forced swimming rats.

As the other possible mechanim, anti-fatigue effect of panaxydol may regulate via attenuation of oxidative stress. It is known that oxidative stress occurs following FST and subsequently may lead to pathology and clinical symptoms of fatigue [34-36]. Bao L et al., has shown that ginseng improved mitochondrial functions and inhibited oxidative stress in skeletal muscles of mice after the FST which may be an action pathway of its anti-fatigue effects [11]. Therefore, panaxydol may have an anti-fatigue effect by reducing the level of oxidative stress indicators such as superoxide dismutase or malondialdehayde in forced swimming model. Further studies should be performed to confirm the mechanisms underlying the anti-fatigue effect of panaxydol on forced swimming-induced fatigue.

5. Conclusion

In present study, the forced swimming time in cWG distilled extract-treated group was significantly longer than that of control group on day 4 and 5. Panaxydol-treated groups showed significantly enhanced performance in the forced swimming, compared to control. In addition, a significant decrease in serum LDH level was found in panaxydol-treated group, while there were no alternations in levels of serum BUN and LAC and glycogen in liver or soleus muscle. Taken these results, distilled extract of cultivated wild ginseng and its active component panaxydol produce anti-fatigue activity by reducing activity of LDH in the rat forced swimming model. some pentacyclic triterpenoids.

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Availability of data and materials

The datasets used and analyzed during the current study available from the corresponding authors (Hee Young Kim, hykim@dhu.ac.kr; Hwa-Seung Yoo, altyhs@dju.kr) on reasonable request.

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