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# The Bioactivity of Natural Product in the Ovariectomized Rat

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**Abstract** To investigate the deaging effects of intraperitoneally injected Chondroitin Sulfate (CS) on various enzyme activity (AST, ALT, MDA (Malon dialdehyde), SOD (Superoxide dismutase), Catalase, GSH (reduced-glutathione), GSSG (oxidized-glutathione), GPx (Glutathione peroxidaes)) and histopathology of liver tissue, ovariectomized rats were used. The antioxidative effects of chondroitin sulfate (100 mg/kg and 200 mg/kg body weight) were investigated at the antioxidative enzyme activities of liver homogenate fractions (liver total homogenate, mitochondrial, and microsomal fractions) and sera. In addition, the rat liver was histologically examined. Intraperitoneally injected CS, depend on dosage, indicated a protective effect against ovariectomy-induced aging. Moreover, inflammation and cirrhosis in liver tissue of CS treated group were significantly decreased. Based on these results, intraperitoneally injected CS is a useful material to delay aging.

**Key words**: Chondroitin Sulfate, Ovariectomy, Mitochondrial fraction, Microsomal fraction, Antioxidative enzyme

## Introduction

The aged rat model of ovarian hormone deficiency-induced ovariectomy is a practical, convenient, and cost-effective animal model of the prevention and treatment of ovarian hormone deficiency. Chonroitin Sulfate (CS) is a component of articular cartilage proteoglycan and plays an important role in the elasticity and function of the articular cartilage [1]. Reduced cartilage chondroitin sulfate levels may be risk factor involved in articular disorders in elderly people [2]. CS has many biological responses in its structure. CSs are heteropolysaccharides composed of alternate sequences of differently sulfated residues of uronic acid ($\beta$-D-glucuronic) and $\alpha$-d-N-acetylgalactosamine linked by $\beta$-(1→3) bonds. Because of its structural characteristics, it gave me a impression that it might be a biological response modifier or a radical scavenger. Especially, microsomal fraction has major role of xenobiotic metabolic activation (most of xenobiotics in microsomal metabolism require NADPH as electron transfer structure. Increased lipid peroxidation of microsomal lipid catalized by NADPH reduces capacity of xenobiotic metabolism [3-5].). And, mitochondrial fraction has essential role of substance and energy metabolism. In order to elucidate the protection mechanism of CS, the following parameters were examined in this study. Aging of rats induced by ovariectomy was examined in various enzyme activities and histopathology of liver tissue.

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## MATERIALS AND METHODS

### Animals and Sample Collection

Sparague-Dowley (SD) rats (8 weeks old female, 130 g~150 g) were supplied from Korean Experimental Animal Center (KCAC), and acclimated for 7 days. All animals were maintained in seperated cages with laboratory chow and tap water *ad libitum*. During the experiment, the animals were housed at 22±1℃ and 60±5% relative humidity with a 12 hour light/dark cycle. Body weight was measured daily. A total of 28 SD rats were divided into 4 groups : non-operation group (Sham), ovariectomized group (OVX) and dose-dependent CS groups (100 mg/kg (OVX+CS 100), 200 mg/kg (OVX+CS 200)) after ovariectomy. Animals were ectomized both ovaries. After 2 days, CS was injected intraperitoneally by 100 mg/kg and 200 mg/kg. Physiological saline was injected into the rest of animals. At the fifteenth weeks, animals were anesthesized with ether and dissected. Blood and liver were obtained for further analysis. The blood samples were then centrifuged at 3000 rpm for 10 min at 4℃ to obtain serum samples, then stored -80℃. The liver were rinsed saline solution, then also stored at -80℃. Liver tissues were homogenized in 1:5 volumes of PBS (pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min. And then, the supernatant was used as liver total homogenated sample. The seperation of mitochondria and microsome frac-

tions described by Ha [6] were used.

### Analytical Pocedures

**AST and ALT :** AST (Aspartate transaminase) and ALT (Alanine transaminase) were extracted from sera by using the method of Reitman-Frankel [7]. AST and ALT concentrations were determined enzymatically by using commercially available reagents (AST kit no. BC101-O and ALT kit no. BC101-P, Young Dong, Korea).

**Protein assay :** Protein concentration was measured using a modified assay originally discribed by Lowry [8].

**MDA :** Liver malondialdehyde (MDA) levels were measured as described previously [6].

**SOD :** SOD activity was measured by using a modified assay originally described by Fridovich [9].

**Catalase :** CAT (catalase) activity was measured using the method of Aebi [10].

**GSH and GSSG :** Liver GSH levels were measured as slightly modified method described by Elman [11]. On the other hand, liver GSSG levels were measured using a modified assay discribed by Racker [17].

**GPx avtivity :** Liver GPx activities were estimated by the method of Lawrence and Burk [13].

**Histological examination on the liver tissue :** The liver tissue was fixed for overnight in 10% formaldehyde solution (dissolved in phosphate buffer (pH7.4)), and then changed ranging from 70% alcohol to 100% alcohol consecutively. After, it was changed from 100% alcohol to xylene, and then embedded in paraffin wax, sectioned in 5 ㎛ size, followed by staining with hematoxylin-eosin (H&E).

### Statistical analysis

The data was subjected to analysis of variance followed by student's Test to determine which means were significantly different from each other or controls. In all cases, a P value of $< 0.05$ was used to determine significance.

## RESULTS AND DISCUSSIONS

### AST and ALT activities

Fig. 1 shows the effects of CS on serum AST and ALT from ovariectomized rats. There were significant changes following administration two different doses of CS. In that study, AST activity in OVX group (102.38 unit/㎖) was found to be significantly higher than in Sham group (48.61 unit/㎖). OVX+CS100 (79.28 unit/㎖) and OVX+CS200 (66.72 unit/㎖) groups were found to be significantly lower than OVX group. Both OVX+CS100 and OVX+CS200 groups in serum AST activity were reduced to 42% and 66% compared with NO group, respectively. On the other hand, ALT activity in the OVX group (29.05 unit/㎖) was measured similar to the Sham group (30.45 unit/㎖). ALT activity in the OVX+CS100 (34.21 unit/㎖) and OVX+CS200 (33.8 unit/㎖) groups were determined similar to OVX group. Therefore, increased AST activity in the OVX group may

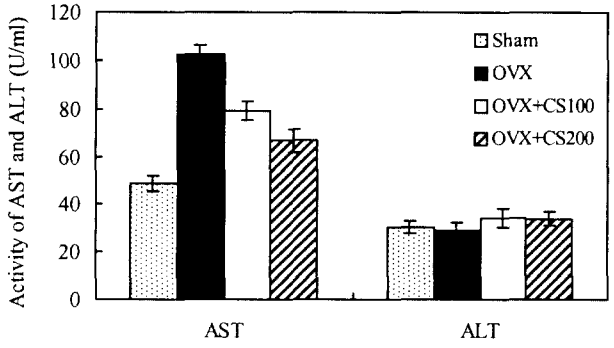


**Fig. 1.** Effect of CS on AST and ALT in ovariectomy induced aging rat
Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Ovariectomy + Chondroitin sulfate (100 mg/kg body weight, i.p.)
OVX+CS200 : Ovariectomy + Chondroitin sulfate (200 mg/kg body weight, i.p.)

be attributed to generation of more liver necrosis than CS groups, while ALT activities in all group were subjected to normal range.

### MDA level

The localization of radical formation resulting in lipid peroxidation, measured as malondialdehyde (MDA) in rat liver homogenate is shown in Fig. 2. MDA contents in the **liver total homogenate** were increased in the OVX group (9.10 nmol/mg protein) compared to Sham group (6.32 nmol/mg protein). MDA level of the OVX+CS100 group (7.19 nmol/mg protein) was inhibited by 69% compared to OVX group. And, MDA level of the OVX+CS200 group (6.72

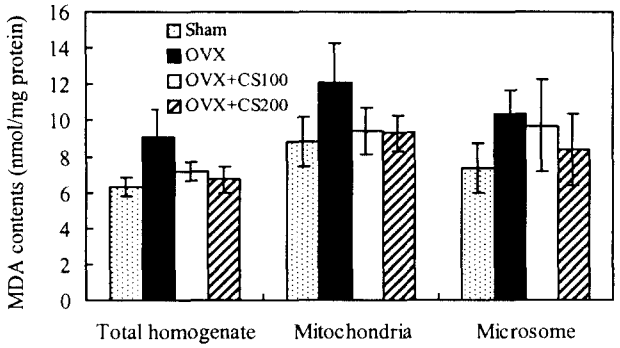


**Fig. 2.** Inhibition percentage MDA contents in the liver total homogenate, mitochondrial and microsomal fractions by CS in ovariectomy induced aging rat
Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Ovariectomy+Chondroitin sulfate(100 mg/kg body weight, i.p.)
OVX+CS200 : Ovariectomy+Chondroitin sulfate(200 mg/kg body weight, i.p.)

nmol/mg protein) was inhibited by 86%. **Mitochondrial MDA contents** in the mitochondrial fraction were increased in OVX group (12.12 nmol/mg protein) compared to Sham group (8.84 nmol/mg protein). The mitochondrial MDA level of OVX+CS100 group (9.41 nmol/mg protein) was inhibited by 83%. Significantly, microsomal MDA level of OVX+CS200 group (9.30 nmol/mg protein) was inhibited by 86%. MDA level in the **microsomal fraction** was increased in OVX group (10.39 nmol/mg protein) compared to Sham group (7.35 nmol/mg protein). Microsomal MDA contents of OVX+CS100 group. (9.72 nmol/mg protein) was inhibited by 22%. Remarkably, microsomal MDA contents of OVX+CS200 group (8.37 nmol/mg protein) were inhibited by 66%. In these results, the contents of MDA in liver total homogrnate and each fractions were significantly decreased in CS groups than NO group.

## SOD activities

The effects of CS on SOD activity in liver each fractions are shown in Fig. 3. SOD activity of the **liver total homogenate** in OVX group (0.16 mU/mg protein) was found to be lower than in Sham group (35.41 mU/mg protein). OVX+CS100 (2.95 mU/mg protein) and OVX+CS200 (3.17 mU/mg protein) in the SOD activities had synergism of 8% and 9%, respectively. **Mitochondrial SOD activity** in OVX group (0.31 mU/mg protein) was examined to be lower than in Sham group (29.11 mU/mg protein). Mitochondrial SOD activities in OVX+CS100 (6.17 mU/mg protein) and OVX+CS200 (13.84 mU/mg protein) groups were observed to be higher than in OVX group. SOD activities of OVX+CS100 and OVX+CS200 groups in mitochondrial fractions had synergism of 20% and 47%, respectively. **Microsomal SOD activity** in OVX group (0.11 mU/mg protein) was determined to be lower than Sham group (0.19 mU/mg protein). Microsomal SOD activities of OVX+CS100 (0.17 mU/mg protein) and OVX+CS200 (0.19 mU/mg protein) groups were examined to be higher than OVX group. SOD activities of OVX+CS100 and OVX+CS200 groups in microsomal fraction had synergism of 75% and 100%, respectively. The highest activity could be observed in the mitochondrial fraction of OVX+CS200 group and the highest SOD activity recovery was shown in microsomal fraction of CS treated group. Other authers also report the increase in antioxidant injected liver SOD observed in this study [14-17]. The decrease of SOD activities might be connected with increased lipidperoxide and free radical.

## Catalase levels

Catalase activities in the liver total homogenate and each fractions are shown in Fig. 4. Catalase activity of **liver total homogenate** in OVX group (317.03 mU/mg protein) was found to be conspicuously lower than in Sham group (415.61 mU/mg protein). Total homogenated liver catalase activity in OVX+CS100 (376.31 mU/mg protein) and OVX+CS200 (392.17 mU/mg protein) groups were examined to be higher than OVX group. Catalase activities of OVX+CS100 and OVX+CS200 groups in liver total homogenate had synergism of 60% and 76%, respectively. **Catalase activity of mitochondrial fraction** in OVX group (452.46 mU/mg protein) was meaured to be strikingly lower than in the Sham group (706.19 mU/mg protein). **Microsomal catalase activities** in OVX+CS100 (483.68 mU/mg protein) and OVX+CS200 (610.86 mU/mg protein) groups were determined to be higher than Sham group. Catalase activities of OVX+CS100 and OVX+CS200 groups in microsomal fraction had synergism of 12% and 62%, respectively. Microsomal

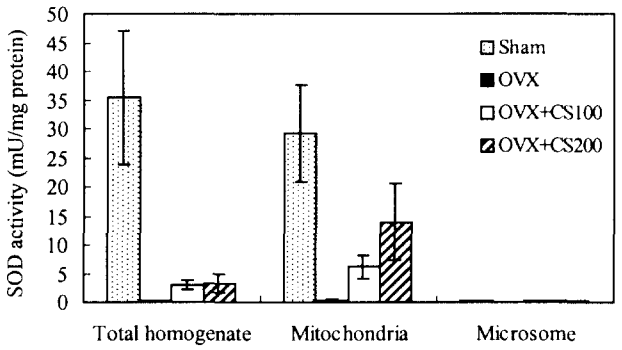


**Fig. 3.** SOD activity following treatment with CS in liver total homogenate, mitochondrial and microsomal fractions of ovariectomy induced aging rat
Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Ovariectomy+Chondroitin sulfate (100 mg/kg body weight, i.p.)
OVX+CS200 : Ovariectomy+Chondroitin sulfate (200 mg/kg body weight, i.p.)

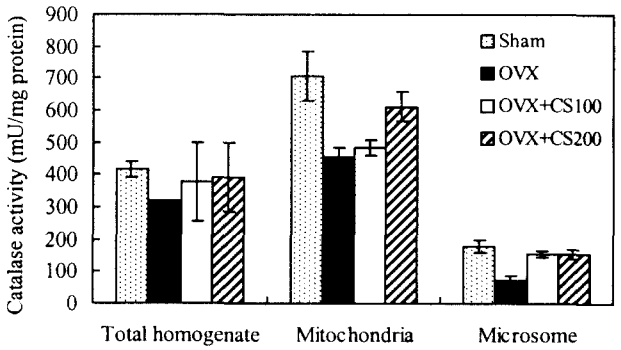


**Fig. 4.** Catalase activity following treatment with CS in liver total homogenate, mitochondrial and microsomal fractions of ovariectomy induced aging rat
Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Ovariectomy+Chondroitin sulfate (100 mg/kg body weight, i.p.)
OVX+CS200 : Ovariectomy+Chondroitin sulfate (200 mg/kg body weight, i.p.)

catalase activity in OVX group (74.94 mU/mg protein) was examined to be lower than in the Sham group (177.8 mU/mg protein). Microsomal catalase activities in OVX+CS100 (154.38 mU/mg protein) and OVX+CS200 (155.8 mU/mg protein) groups had synergism of 77% and 79%, respectively. OVX+CS200 group revealed the highest catalase activity in the mitochondrial fraction and the highest catalase recovery effect of 79 percent in microsomal fraction. The increase of catalase activities might be coincided with decreased lipidperoxide and free radical.

## GSH · GSSG levels

GSH level of liver total homogenate in OVX group (18.19 nmol/mg protein) was found to be lower than in Sham group (23.25 nmol/mg protein). GSH level of OVX+CS100 group in liver total homogenate was pronounced with 20.58 nnmol/mg protein. These rsults showed the synergism of 47%. Also, GSH level of OVX+CS200 group (22.99 nmol/mg protein) had the synergism of 94%. GSH level of mitochondrial fraction in Sham group (2.84 nmol/mg protein) was mearured to be higher than in OVX group (2.13 nmol/mg protein). Mitochondrial GSH level of OVX+CS200 group (2.75 nmol/mg protein) was determined to be slightly higher than OVX+CS100 group (2.28 nmol/mg protein). Mitochondrial GSH level of OVX+CS100 and OVX+CS200 groups had synergism of 21% and 87%, respectively. GSH level of microsomal fraction in OVX group (0.48 nmol/mg protein) was found to be lower than in Sham group (2.05 nmol/mg protein). Microsomal GSH level was increased by 64% and 71% in OVX+CS100 (1.49 nmol/mg protein) and OVX+CS200 (1.59 nmol/mg protein) groups, respectively. GSSG level of liver total homogenate in OVX group (3.41 nmol/mg protein) was found to be higher than in Sham group (2.84 nmol/mg protein). GSSG level of OVX+CS100 group in liver total homogenate was determined to 3.12 nmol/mg protein. This value was higher than in OVX+CS200 group (3.05 nmol/mg protein). These results showed that GSSG level of OVX+CS100 and OVX+CS200 groups in liver total homogenate had 51% and 63% of inhibition effects, respectively. Mitochondrial GSSG level in OVX group (4.52 nmol/mg protein) was measured to be higher than in Sham group (3.38 nmol/mg protein). Mitochondrial GSSG level of OVX+CS100 (3.72 nmol/mg protein) and OVX+CS200 (3.54 nmol/mg protein) groups had 70% and 86% of inhibitory effects, respectively. Microsomal GSSG level in OVX group (4.40 nmol/mg protein) was examined to be hugher than in Sham group (3.89 nmol/mg protein). The inhibitory effect of OVX+CS100 group (4.03 nmol/mg protein) was 73%. Also, the OVX+CS200 (4.01 nmol/mg protein) had 76% of inhibitory effect. GSSG level displayed the highest inhibition rate of 86 percent in mitochondrial fraction of OVX+CS200 group but the lowest rate of 51 percent in total liver homogenate of OVX+CS100 group. The decrease in GSSG level observed in CS groups resulted from decreasing cell injury than in the OVX group. On the other hand, GSH/GSH+GSSG amount was the highest in total liver homogenate fraction of OVX+CS200 group among the material injected groups, as shown in Table 1. It is thought that the amount of GSH/GSH+GSSG decreased in OVX group but increased in OVX+CS group dose-dependently as CS was injected, which means CS reduced GSH consumption.

## GPx levels

The GPx activities of the liver homogenate and each fractions on ovariectomized rat are shown in Fig. 5. GPx activity of liver total homogenate in OVX group (136.09 mU/mg protein) was found to be lower than in Sham group (162.3 mU/mg protein). GPx activity of OVX+CS100 group in liver total homogenate was pronounced with 143.74 mU/mg protein. These rsults showed the synergism of 29%. Also, GPx activity of OVX+CS200 group OVX+CS200 group indicated the highest GSH level of 94 percent in total liver homogrnate, while OVX+CS100 group showed the lowest GSH level of 21 percent in mitochondrial fraction (159.44 mU/mg protein) had the synergism of 89%. GPx activity of mitochondrial fraction in Sham group (78.69 mU/mg protein)

**Table 1.** The level of GSH/GSH+GSSG in the liver total homogenate and fractions of ovariectomized rat

| Experimental group | Total homogenate | Mitochondrial fraction | Microsomal fraction |
|---|---|---|---|
| Sham(7) | 0.89 | 0.46 | 0.35 |
| OVX(7) | 0.84 | 0.32 | 0.10 |
| OVX+CS100(7) | 0.87 | 0.38 | 0.27 |
| OVX+CS200(7) | 1.15 | 0.44 | 0.28 |

Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Chondroitin sulfate (100 mg/kg body weight, i.p.)

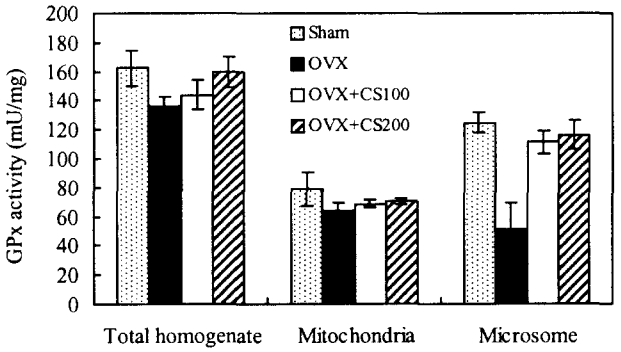


**Fig. 5.** GPx activity following treatment with CS in liver total homogenate, mitochondrial and microsomal fractions of ovariectomy induced aging rat
Sham : Normal Control
OVX : Ovariectomy control
OVX+CS100 : Ovariectomy+Chondroitin sulfate (100 mg/kg body weight, i.p.)
OVX+CS200 : Ovariectomy+Chondroitin sulfate (200 mg/kg body weight, i.p.)

was mearured to be higher than in OVX group (64.26 mU/mg protein). Mitochondrial GPx activity of OVX + CS200 group (70.41 mU/mg protein) was determined to be higher than OVX + CS100 group (68.40 mU/mg protein). Mitochondrial GPx activity of OVX + CS100 and OVX + CS200 groups had synergism of 29% and 43%, respectively. GPx activity of microsomal fraction in OVX group (50.92 mU/mg protein) was found to be lower than in Sham group (124.91 mU/mg protein). Microsomal GPx activity was increased by 82% and 89% in OVX + CS100 (111.46 mU/mg protein) and OVX + CS200 (116.48 mU/mg protein) groups, respectively.

### Effect of CS in liver tissue section

Serum biological enzyme activity and tissue MDA content in chondroitin sulfate treated groups after ovariectomy were examined. These results indicated that ovariectomy induced aging reduced followed by CS injection significantly. As shown in Fig. 6, liver tissue of Sham group (Fig. 6-A) showed no abnormal state in the central and portal veins (PV) while liver tissue of ovariectomy induced aging group (Fig. 6-B) was observed to accumulated lipid and necrosis of surrounded with central vein (CV). On the other hand, inflammation and cirrhosis in liver tissue of CS treated groups after ovariectomy were significantly decreased (Fig. 6-C, D). It is thought that CS can prevent the ovariectomy induced aging significantly on the basis of histological observation

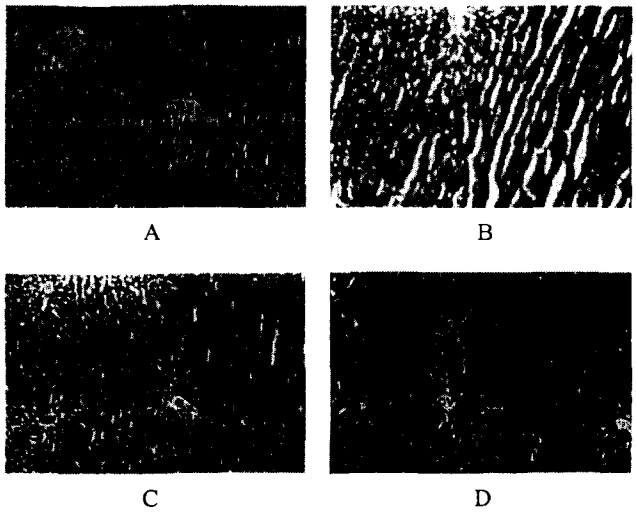


**Fig. 6.** Histopathologic examination of various experimental group in aging (H&E, ×200)
(A) Sham : non-operation
(B) OVX : ovariectomy
(C) OVX + CS100 : ovariectomy + 100 mg/kg of CS
(D) OVX + CS200 : ovariectomy + 200 mg/kg of CS

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