

The Effects of Anti-Inflammatory and Liver Function using Heat-Treated Cabbage

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Abstract

The cabbage extract of the research does not show cytotoxicity, and thus can be used safely. In an experiment performed on an animal model with liver injury induced by a drug (APAP), it could be seen that the cabbage extract exhibited the effects of protecting liver and improving liver function by effectively reducing AST and ALT which are liver injury markers, indicating that the cabbage extract is effective as a pharmaceutical composition for preventing or treating liver disease. In particular, the cabbage extract was effective in treating inflammation of the liver by reducing the expression of the inflammatory mediators iNOS and COX-2 and the proinflammatory cytokine IL-1 β , which are involved in acute inflammatory reactions accompanying liver injury. In the research, an extract of cabbage heat-treated at a temperature of 100 to 150°C had a better liver function-improving effect or anti-inflammatory effect than an extract of raw cabbage.

Keywords: *Anti-inflammatory, Cytotoxicity, Cabbage, Acetaminophen, Hepatoprotective*

1. Introduction

The liver is one of the important organs of the human body, which is responsible for body's metabolism which appropriately changes ingested foods into nutrients required by various tissues and processes waste products remaining after use in tissues. Specifically, the liver secretes bile, a digestive fluid, metabolizes proteins, carbohydrates and fats, stores glycogen, fat-soluble vitamins, and other substances, synthesizes blood-clotting factors, removes wastes and toxins from the blood, regulates blood volume, and destroys old red blood cells [1-3]. Various factors, including mental stress, excessive intake of fatty food or alcohol, viral infection, drugs or smoking, exposure to harmful substances such as pollutants, and malnutrition, may cause hepatic dysfunction. In addition, hepatic dysfunction interferes with body's defense and detoxification mechanisms, causing abnormalities in the immune system and causing other diseases. Hepatic dysfunction causes various symptoms such as weakness, hypotension, frequent contusion and hemorrhage, delirium tremens, emotional dullness, electroencephalographic changes, and intraabdominal fluid accumulation. Acetaminophen (APAP), a major component of Tylenol, is a generic drug used worldwide for the treatment

of fever and pain. It is relatively safe when used at therapeutic concentrations [4]. However, when an excessive amount of APAP is ingested it may cause side effects such as hepatic necrosis, neurotoxin and cirrhosis, and can lead to death in severe cases. Recently, it has been reported that when an excessive amount of APAP is administered to a living body, it enters a large amount of leukocytes, causing acute inflammation. In other words, proinflammatory cytokines, such as interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), interleukin-1 and IL-6, which are well-known cytokines that cause inflammatory responses, are produced in large amounts within 10 hours and accelerate inflammatory responses. Furthermore, inflammatory mediators, such as cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), are induced by APAP and cause fatal damage to the living body. It has been reported that patients with liver diseases caused by APAP reach about 10% and the toxicity of APAP is more serious in children than adults and more serious in alcohol eaters than non-alcohol eaters. NAC (N-acetylcysteine) is used for the treatment of hepatotoxicity caused by APAP, but it has been reported that NAC is difficult to administer orally due to its very unpleasant odor and taste and the administration of NAC by intravenous injection can cause anaphylactic shock. In order to diagnose various liver-related diseases, it is necessary to comprehensively perform several biochemical tests. Several test items for this purpose are collectively referred to as liver function tests. Major test items include AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), GGT (gamma-glutamyl-transferase), and bilirubin. In addition to these test items, items such as total protein, albumin, LDH (lactate dehydrogenase), ammonia and the like may also be tested. Among them, AST (GOT) and ALT (GPT) are enzymes present in hepatocytes and are released into the blood mainly when hepatocytes are damaged. Thus, blood AST and ALT levels can be used as markers of liver damage. In the early stages of acute hepatocyte injury, the level of AST which is present at a higher concentration in hepatocytes increases compared than the level of ALT, but after 24 to 48 hours and in chronic hepatocyte injury, the level of ALT with a longer half-life generally further increases. In alcoholic hepatitis, AST further increases. Meanwhile, since the liver is an organ having a large buffer capacity, liver disease symptoms generally do not appear in the initial stage of the disease, and since the amount of pain-feeling nerves in the liver is small, liver disease symptoms are generally found after the disease has worsened considerably. Liver cirrhosis or liver cancer is the last stage to which various liver diseases commonly lead when progressing to a chronic stage. The liver is an organ whose initial care is very important. Accordingly, these studies have been conducted to provide hepatoprotective effect and anti-inflammatory of natural substances that can be used safely [5-7].

2. Experiment Materials and Methods

2.1 Preparation of Heat-Treated Cabbage Extract(HCE)

Cabbage purchased from an agricultural and marine products market was washed, cut to a size of about 0.5 cm \times 0.5 cm \times 0.5 cm, and then freeze-dried. The freeze-dried sample was placed in the inner chamber of a heat-treatment apparatus (Jisco, Seoul, Korea), which was designed and manufactured to be capable of resisting even a pressure of 10 kg/cm² or higher. Water was placed in the outer chamber, and the sample was heat-treated at a temperature of 140 to 150°C for 6 hours. The apparatus could prevent the sample from coming into direct contact with water and also prevent the carbonization of the sample by direct heat transfer, due to water contained in the outer chamber. The heat-treated sample was cooled, and then crushed using a crusher, and a 10-fold volume (v/v) of distilled water was added, followed by extraction at 60°C for 2 hours. The extract was filtered, and then freeze-dried before use [8,9].

2.2 Evaluation of Cytotoxicity

To measure cytotoxicity, RAW 264.7 cells were dispensed in each well of a 96-well plate at a concentration

of 5×10^3 cells/100 μ L. The RAW 264.7 cells were obtained from the Korean Cell Line Bank and cultured with Dulbecco's Modified Eagle's Medium (DMEM) (supplemented with 5% fetal bovine serum (FBS) and 100 IU/mL penicillin and 100 μ g/mL streptomycin (P/S)) at 37°C under 5% CO₂. Each of the cabbage extract and heat-treated cabbage extracts was added to the dispensed cells at a concentration of 125, 250, 500 or 1,000 μ g/ml and incubated for 24 hours, and then the viability of the cells was measured by MTT assay [10,11].

2.3 Evaluation of Hepatoprotective Effect of a Cabbage Extract

6-8-week-old male BALB/c mice (weighing 19 to 22 g) purchased from Dongyang Biotechnology (Gyeonggi-do, South Korea) were acclimated in a pathogen-free facility under constant condition (temperature: $21 \pm 2^\circ\text{C}$; relative humidity: $60 \pm 10\%$; 12-hr light/12-hr dark cycle) before use. During the experimental period, the animals were allowed to access water and food ad libitum. The animals were divided into the following groups, each consisting of 6 animals: a normal group (untreated group), a negative control group (treated with APAP alone), a positive control group (treated with APAP + NAC), and a sample-administered group (treated with APAP + cabbage extract). The positive control group was orally administered with NAC, known to have the effect of treating APAP-induced hepatotoxicity, at a daily dose of 75 mg/kg for 7 days, and the sample-administered group was orally administered with 500 mg/kg of the heat-treated cabbage extracts at a daily dose of 500 mg/kg. At 2 hours after the last oral administration of NAC or the sample to the positive control group and the sample-administered group, APAP for inducing hepatotoxicity was intravenously administered to all the groups excluding the normal group at a concentration of 400 mg/kg. 24 Hours after administration of the sample, blood was collected through the tail vein, and ALT and AST levels in the collected blood were measured by an assay kit (Asan Pharmaceutical) using a substrate-enzyme reaction [12].

2.4 Evaluation of Anti-Inflammatory Effect of Cabbage Extract

Since liver injury generally causes acute inflammation, whether the cabbage extract would have an anti-inflammatory effect was examined. To this end, RAW 264.7 cells were dispensed in each well of a 6-well plate at a concentration of 5×10^5 cells/100 μ L, and then treated with 0.1 μ g/ml of LPS. At 30 minutes after LPS treatment, the cells were treated with 1 mg/ml of the cabbage extract (CE) or the heat-treated cabbage extract (HCE) and incubated for 18 hours. Next, RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's manual [13]. Thereafter, the expression levels of proinflammatory mediators and cytokines were measured by qRT-PCR using the primers shown in Table 1 below.

Table 1. Primer Sequence.

Gene	Primer sequence	Sequence Number
GAPDH	F: 5'-CACTCACGGCAAATTCACGGCAC-3'	Sequence No 1
	R: 5'-GACTCCACGACATACTCAGCAC-3'	Sequence No 2
iNOS	F: 5'-CCCTTCCGAAGTTTCTGGCAGCAGC-3'	Sequence No 3
	R: 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3'	Sequence No 4
COX-2	F: 5'-CACTACATCCTGACCCACTT-3'	Sequence No 5
	R: 5'-ATGCTCCTGCTTGAGTATGT-3'	Sequence No 6
TNF- α	F: 5'-TTGACCTCAGCGCTGAGTTG-3'	Sequence No 7
	R: 5'-CCTGTAGCCCACGTCTAGC-3'	Sequence No 8

IL-1 β	F: 5'-CTGTGGAGAAGCTGTGGCAG-3'	Sequence No 9
	R: 5'-GGGATCCACACTCTCCAGCT-3'	Sequence No 10
1L-6	F: 5'-GTACTIONCAGAAAGACCAGAGG-3'	Sequence No 11
	R: 5'-TGCTGGTGACAACCACGGCC-3'	Sequence No 12

2.5 Statistical analysis

Data are presented as mean \pm SEM. One-way ANOVA and Dunnett's test were applied for the statistical evaluation of the data. Differences with *** $p < 0.001$ were considered significant. Respective significant marks other than the above mentioned are described in the figure legends.

3. Result and Discussion

3.1 Cell viability of a cabbage extract

Figure 1 is a graph showing the results of measuring the cell viability. As can be seen therein, both the cabbage extract and the heat-treated cabbage extract showed no cytotoxicity, indicating that these extracts can be used safely.

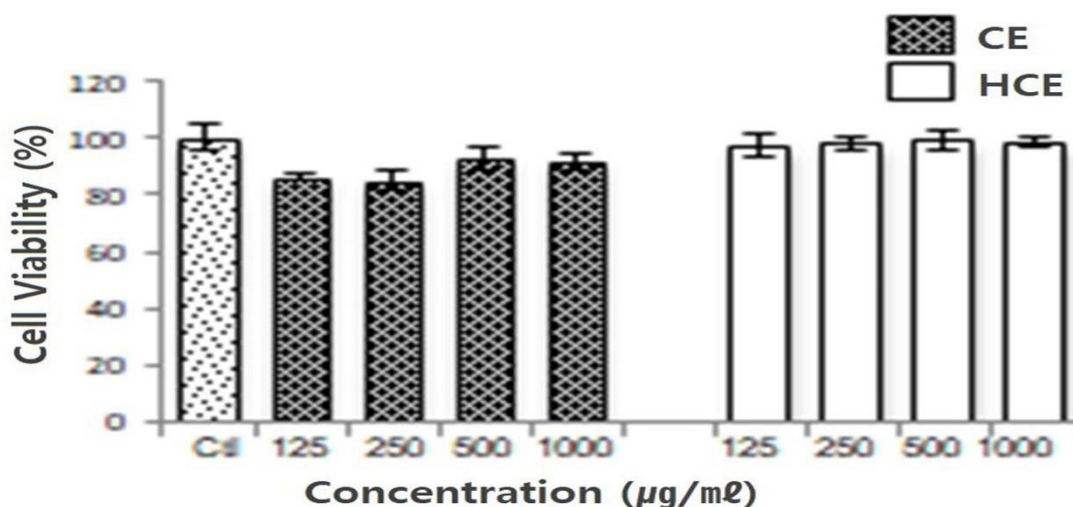


Figure 1. The cell viability effect of the heat-treated cabbage extract. Sample was added to the dispensed cells at a concentration of 125, 250, 500 or 1,000 $\mu\text{g/ml}$ and incubated for 24 hours, and then the viability of the cells was measured by MTT assay. CE; Cabbage extract, HCE; Heat-treated cabbage extract.

3.2 Liver function (ALT and AST) improving activity of a cabbage extract

In order to diagnose various liver-related diseases, it is necessary to comprehensively perform several biochemical tests. Several test items for this purpose are collectively referred to as liver function tests. Major test items include AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), GGT (gamma-glutamyltransferase), and bilirubin. In addition to these test items, items such as total protein, albumin, LDH (lactate dehydrogenase), ammonia and the like may also be tested. Among them, AST (GOT) and ALT (GPT) are enzymes present in hepatocytes and are released into the blood mainly when hepatocytes are damaged. Thus, blood AST and ALT levels can be used as markers of liver damage. In the early stages of acute hepatocyte injury, the level of AST which is present at a higher concentration in

hepatocytes increases compared than the level of ALT, but after 24 to 48 hours and in chronic hepatocyte injury, the level of ALT with a longer half-life generally further increases. In alcoholic hepatitis, AST further increases. Meanwhile, since the liver is an organ having a large buffer capacity, liver disease symptoms generally do not appear in the initial stage of the disease, and since the amount of pain-feeling nerves in the liver is small, liver disease symptoms are generally found after the disease has worsened considerably. Liver cirrhosis or liver cancer is the last stage to which various liver diseases commonly lead when progressing to a chronic stage. Figure 2 and 3 show the results of measuring ALT and AST levels, respectively, in the blood. As can be seen therein, the cabbage extract is effective against APAP-induced liver injury. In particular, the cabbage extract had a significant effect against the expression of AST, suggesting that the cabbage extract is more effective against acute liver injury.

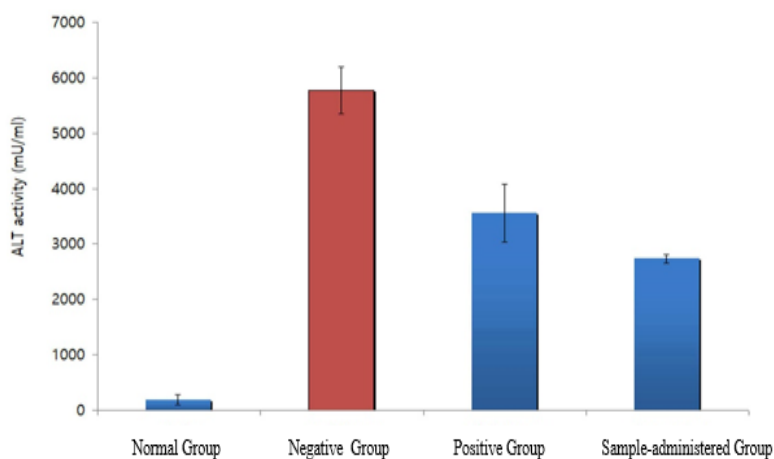


Figure 2. The liver function improving activity (ALT) of the heat-treated cabbage extract. The animals were divided into the following groups, each consisting of 6 animals: a normal group (untreated group), a negative control group (treated with APAP alone), a positive control group (treated with APAP + NAC), and a sample-administered group (treated with APAP + cabbage extract). ***indicates $p < 0.05$ as compared to the control group, ** indicates $p < 0.001$ compared to acetaminophen, and * indicates $p < 0.01$ as compared to the positive control, NAC.

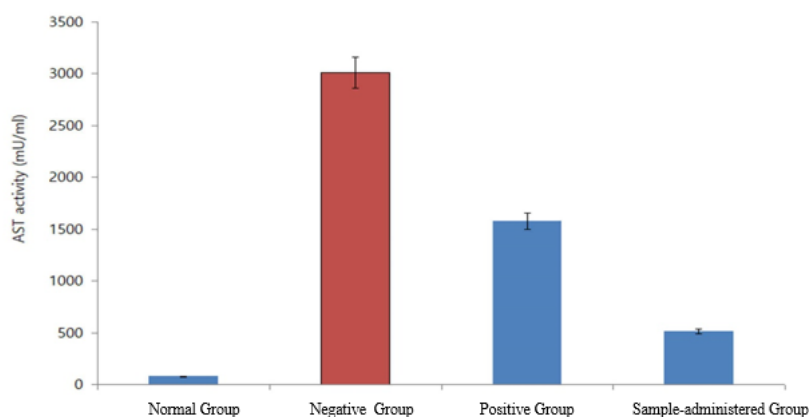


Figure 3. The liver function improving activity (AST) of the heat-treated cabbage extract. The animals were divided into the following groups, each consisting of 6 animals: a normal group (untreated group), a negative control group (treated with APAP alone), a positive control group (treated with APAP + NAC), and

a sample-administered group (treated with APAP + cabbage extract). ***indicates $p < 0.05$ as compared to the control group, ** indicates $p < 0.001$ compared to acetaminophen, and * indicates $p < 0.01$ as compared to the positive control, NAC.

3.3 Evaluation of Hematoxylin & Eosin (H&E)

APAP induced severe hepatocellular damage like, the hepatic lobules showed extensive centrilobular coagulative necrosis with increased eosinophilia. Severe hemorrhage was observed mostly in the hepatic lobule. The sinusoids were dilated and endothelium of the central veins was destroyed. The centrilobular hepatocytes showed severe ballooning degeneration. The sinusoids were heavily congested with red blood cells and lymphocytes. The cell boundaries were ill defined and most nuclei were darkly stained. The amount of heterochromatin increased at the periphery of the nuclei. The nuclei showed extensive karyolysis, pyknosis and karyorrhexis neutrophil accumulation, presence of hemorrhage and parenchymal cell injury as shown by arrows in figure. After blood collection, the mice were sacrificed, and the liver was dissected out, embedded in paraffin, and then stained with H & E. Figure 4 shows staining images of the liver tissue. In Figure 4, A indicates the normal group; B indicates the negative control group; C indicates the positive control group; and D indicates the sample-administered group. As can be seen in Figure 4, serious hepatocyte injury induced by APAP was observed, but this injury was significantly healed by NAC or the cabbage extract.

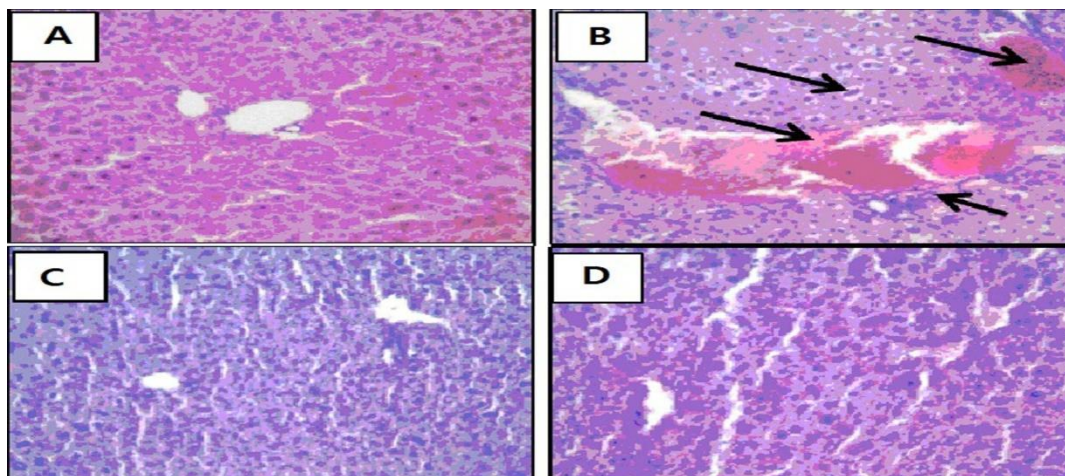


Figure 4. Hematoxylin & Eosin (H&E) staining images of liver tissue of mice in group. (A) control, (B) Negative group, (C) NAC(acetaminophen) and (D) cabbage. Images were taken at a magnification of 20x under a microscope.

3.4 Evaluation of Anti-Inflammatory of Cabbage Extract

As can be seen in Figure 5, the cabbage extract inhibited the expression of the proinflammatory mediators iNOS and COX-2, which was increased by stimulation with LPS, and the expression inhibitory effect of the heat-treated cabbage extract was better than that of the raw cabbage extract. As described above, the heat-treated cabbage extract is prepared using cabbage that has long been used as a food material, and thus it can be used safely in the human body. In addition, it has excellent effects on liver protection and liver function improvement, and thus may be useful as a pharmaceutical composition for preventing or treating liver disease and as a functional health food composition for protecting liver and improving liver function.

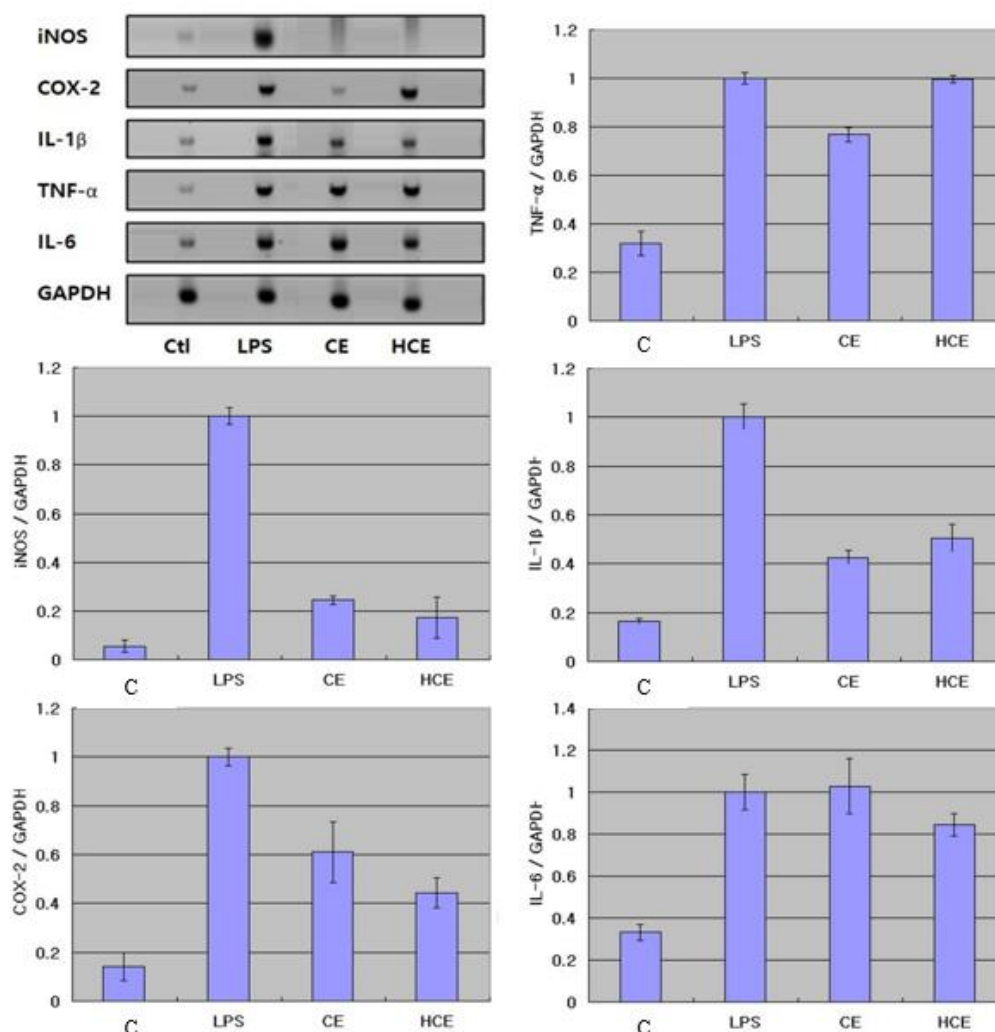


Figure 5. The anti-inflammatory effect of the heat-treated cabbage extract. LPS; LPS-treated, CE; Cabbage extract, HCE; The heat-treated cabbage extract.***indicates $p < 0.05$ as compared to the control group, ** indicates $p < 0.001$ compared to acetaminophen, and * indicates $p < 0.01$ as compared to the positive control, NAC.

4. Conclusion

The cabbage extract of the the research does not show cytotoxicity, and thus can be used safely. In an experiment performed on an animal model with liver injury induced by a drug (APAP), it could be seen that the cabbage extract exhibited the effects of protecting liver and improving liver function by effectively reducing AST and ALT which are liver injury markers, indicating that the cabbage extract is effective as a pharmaceutical composition for preventing or treating liver disease. In particular, the cabbage extract was effective in treating inflammation of the liver by reducing the expression of the inflammatory mediators iNOS and COX-2 and the proinflammatory cytokine IL-1β, which are involved in acute inflammatory reactions accompanying liver injury. In the research, an extract of cabbage heat-treated at a temperature of 100 to 150°C had a better liver function-improving effect or anti-inflammatory effect than an extract of raw cabbage. More specifically, the experimental results relates to a hepatoprotective effects for preventing or treating liver disease

and improving liver function, which comprises a cabbage extract as an active ingredient.

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