Clinical and Experimental Health Sciences

Cynara Scolymus (Artichoke) Improves Liver Regeneration after Partial Liver Resection in Ratsß

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Received: 21.12.2021 **Accepted:** 28.02.2022

ABSTRACT

Objective: Liver regeneration is necessary to restore hepatic mass and functional capacity after partial hepatectomy (PH). Cynara scolymus (CS) is a pharmacologically important plant that contains phenolic acids and flavonoids, and experimental studies have indicated that it has antioxidant and hepatoprotective effects. The aim of this study was to investigate the role of CS in liver regeneration after PH in rats.

Methods: A total of 36 Wistar albino rats weighing 280.5 ± 18.6 g were used. CS leaf extract was administered after partial hepatectomy. The rats were sacrificed at postoperative day 14, and the histological changes were assessed. The mitotic index (MI), nucleus size, hepatocyte size, and binucleation rate (BR) of hepatocytes were assessed using hematoxylin-eosin (H&E) staining.

Results: The rats that received CS extract had significant differences in liver regeneration markers, including the hepatocyte size, mitotic index, and Ki-67 proliferation index ($p<0.05$). The average increase in liver mass in 14 days was higher in the CS group, but the differences between the groups were not significant (1.70±0.2 g in sham group versus 2±0.8 g in CS group, $p=0.75$).

Conclusions: The results indicate that CS leaf extract promoted hepatocellular proliferation and hypertrophy, which resulted in accelerated liver regeneration.

Keywords: Cynara Scolymus, Artichoke, liver regeneration, liver resection, liver histology

1. INTRODUCTION

The liver can regenerate, which allows it to withstand extended hepatectomy. Nevertheless, insufficient remnant liver is one of the most common causes of death after hepatectomy (1). It is well known that insufficient remnant liver size after liver resection or a living donor liver transplant (LDLT) may result in an inability to meet the metabolic demand of the body and can cause liver failure (2).

Liver regeneration is necessary to restore hepatic mass and functional capacity after partial hepatectomy (3). To increase liver regeneration, it is crucial to obtain enough liver mass in order to prevent complications related to liver failure. It has been shown that hepatocyte proliferation is the main factor in liver regeneration (4). It has been reported that intracellular reactions which play role in kinetics of cell proliferation have influences in the liver regeneration (5). Moreover, studies showed that liver regeneration after hepatectomy is regulated by the immune system (6), and the inhibition of lipid peroxidation and increased glutathione peroxidase activation are reported to increase during liver regeneration.

Commonly known as artichoke, *Cynara scolymus* (CS) is a pharmacologically important plant that contains phenolic acids and flavonoids, and experimental studies have indicated that it has antioxidant activity (7). Many studies have shown that CS has liver-protective effects by inhibiting lipid peroxidation and increasing glutathione peroxidase activity (8, 9). Nevertheless, no study has explained the effects of CS on liver regeneration after hepatectomy. The goal of this study was to investigate the effect of CS in liver regeneration after hepatectomy.

2. METHODS

The study was performed using 6-month-old Wistar albino female rats weighing 280.5 ± 18.6 g, which were obtained from Zonguldak Bulent Ecevit University Animal Laboratory. All experimental work related to the study was

DOI: 10.33808/clinexphealthsci.1008534

carried out according to the standards of national guidelines (28914/February 15, 2014). The study was approved by the Ethical Review Board at Zonguldak Bulent Ecevit University, Zonguldak, Turkey (protocol number 2018-12-07/06). The animals were kept in a cage and had free access to food and water. The animals were kept in a day/night cycle at room temperature.

The study was designed using three groups of n=8 rats each, but 36 rats were obtained in case any rat died during hepatectomy or right after. In total, 12 rats died during or right after the procedure. Thus, the study was started with three groups of 8 rats each.

In the control group, rats did not have surgery and received regular standard food and water. In the sham group, rats underwent hepatectomy but received standard regular food and water. In the CS Group, rats underwent hepatectomy and received CS extracts (by oral gavage) in addition to standard food and water.

Partial hepatectomy was performed by the removal of a part of the middle and left lateral lobes of the liver. CS leaf extract was administered at a dose of 0.16 mg/kg/day two times **orally** to rats in the CS group. At postoperative day 14, the rats were sacrificed. By the end of the study, one rat in the control group and two rats in both the sham and CS groups died.

The average liver weight was calculated for each group. The mitotic index (MI), nucleus size, hepatocyte size, and binucleation rate (BR) of hepatocytes were assessed using hematoxylin-eosin (H&E) staining. For morphological evaluation Olympus CX43 model microscope was used. The mitotic activity was determined using a microscope at 10 high-power fields (HPFs) (X40). The sizes of the nuclei and hepatocytes were determined using 3 HPFs. In this study a photograph of each area belonging to a HPF was taken by the camera attachment of confocal microscope. As we know in every HPF nearly 500 cell can be obtained. The microscopic image of each HPF was transferred to the image J analysis program. In this program, the nucleus and cell size were measured individually for each hepatocyte. All values were summed numerically and divided by the total number of cells within the area to calculate the mean value. This calculation was done separately for both nucleus and cell size. Proliferation index for hepatocytes were evaluated by Ki67 immunohistochemistry. Immunohistochemistry was performed with monoclonal ready-to-use antibody against human Ki-67 antigen (Clone 30-9; Ventana, Tucson, AZ, USA). Rabbit Monoclonal Primary antibody. Staining was performed using a Roche Ventana Automatic Device. The proliferation index was calculated based on the evaluation of 100 hepatocytes with positive stained nuclei**.** We accepted brown staining of nucleus of tumor cells as positive for Ki67. The intensity was varying cell to cell. We considered any degree of intensity as positive staining. The negative tumor cells were blue. In this evaluation detecting tumor cells are important. Because tumor cells are mixed with stromal and lymphoid cells. We evaluated the areas with the most intense staining by scanning the areas. These areas were assessed and minimum 500 cells were counted. In these areas positive staining cells were deterimined. Finally Ki67 proliferation indeks was calculated like this; number of positive staining cells/ number of tumor cells x100 (10)

2.1. Statistical Analyses

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) was used for the statistical analyses. Data are expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was applied to test the differences between groups. Significant differences between two groups were determined using a post-hoc Tukey test, and $p < 0.05$ was considered significant.

3. RESULTS

The average size of livers in the control group was 9.92 ±1.3 g, which is considered as an average weight in all groups. The liver index was calculated as a percentage (%) of the rats' liver weight (g) with respect to the body weight (g), which was 3.54%. The average hepatectomy liver weight in the sham and CS groups was 2.2 ±0.7 g, which is 22.2% of the total liver weight. The average size of livers at the end of the study in sham group was 9.41±0.8g (94.9% of the original liver), while that of the CS group was 9.71± g (97.8%). The average increase of liver mass in 14 days was higher in the CS group than the sham group, but the difference was not significant (1.70±0.2 g in sham group versus 2±0.8 g in CS group, p=0.75). No significant difference was found in the liver index in the sham group versus the CS group (3.36% versus 3.46%, p=0.67).

The effect of CS on the histological morphology after hepatectomy was analyzed. The rates of steatosis, inflammation, sinusoidal dilatation, congestion, and hydropic degeneration in liver cells between three groups were compared (Table 1). These findings were assigned grades of 1 of 4 based on the literature (11). H&E staining indicated that steatosis did not occur in any group, but inflammation was slightly higher in both the sham and CS groups. Although there was no differences in sinusoidal dilatation between groups, congestion and the degree of hydropic degeneration were higher in both the sham and CS groups (Table 1).

Table 1. *The histological liver changes in groups*

Groups	Steatosis	Inflammation	Sinusoidal dilatation	Congestion	Hydropic dejeneration
C	0	0	+	+	0
S	0	+	+	++	+
CS	0	+	+	++	+

None: 0 Minimal: + Mild: ++ Moderate: +++ High: ++++

In further comparisons between experimental groups, the changes were significantly different between all three of

them (Table 2). Histological alterations are shown in Figure 1. H&E staining revealed that the mean size of nuclei was significantly higher in the CS group than both the control group (10.4±0.6 vs. 7.75 ±0.2 µm, p<0.0001) and the sham group (10.4±0.6 vs. 8.33±0.75 µm, p=0.0068). The mean size of nuclei was significantly higher in the sham group than the control group (8.33±0.75 vs. 7.75 ±0.2 µm, p=0.18). The mean size of hepatocytes was significantly higher in the CS group than in both the control group (480.6 ±38 µm vs. 315.9±80, p=0.0021) and sham group (480.6 ±38 µm vs. 265.8± 18.9 µm, p=0.0003). However, no significant difference was found between the sham group and control group (315.87±80.4 vs. 265.8± 19 µm, p=0.456).

The mean MI was significantly higher in the CS group than both the control group (4.7±1.2 vs. 2.25±1.3, p=0.015) and sham group (4.7±1.2 vs. 1.5±0.7, p= 0.348)). However, no statistically difference was found between the sham group and the control group (2.25±1.3 vs. 1.5±0.7, p= 0.348). Statistically significant differences were found in the BR in the CS group versus the sham group (29.2±6 vs. 12±5%, p= 0.003) and versus the control group (29.2±6 vs. 6.5 ±2, p=0.0001), as well as between the control group and sham group (12±5% vs. 6.5 ±2.1, p= 0.042)

The Ki-67 proliferation index rate was compared between groups, and the differences were significant (Table 2, Figure 2). The mean rate of the Ki-67 proliferation index was 2% in the control group, while it was 3% in the sham group, but the differences were not significant (2% and 3%, p=0.114). The mean rate of the Ki-67 proliferation index was 8% in the CS group, and there was a significant difference between the CS group and sham group (8 % vs. 3% p=0.043).

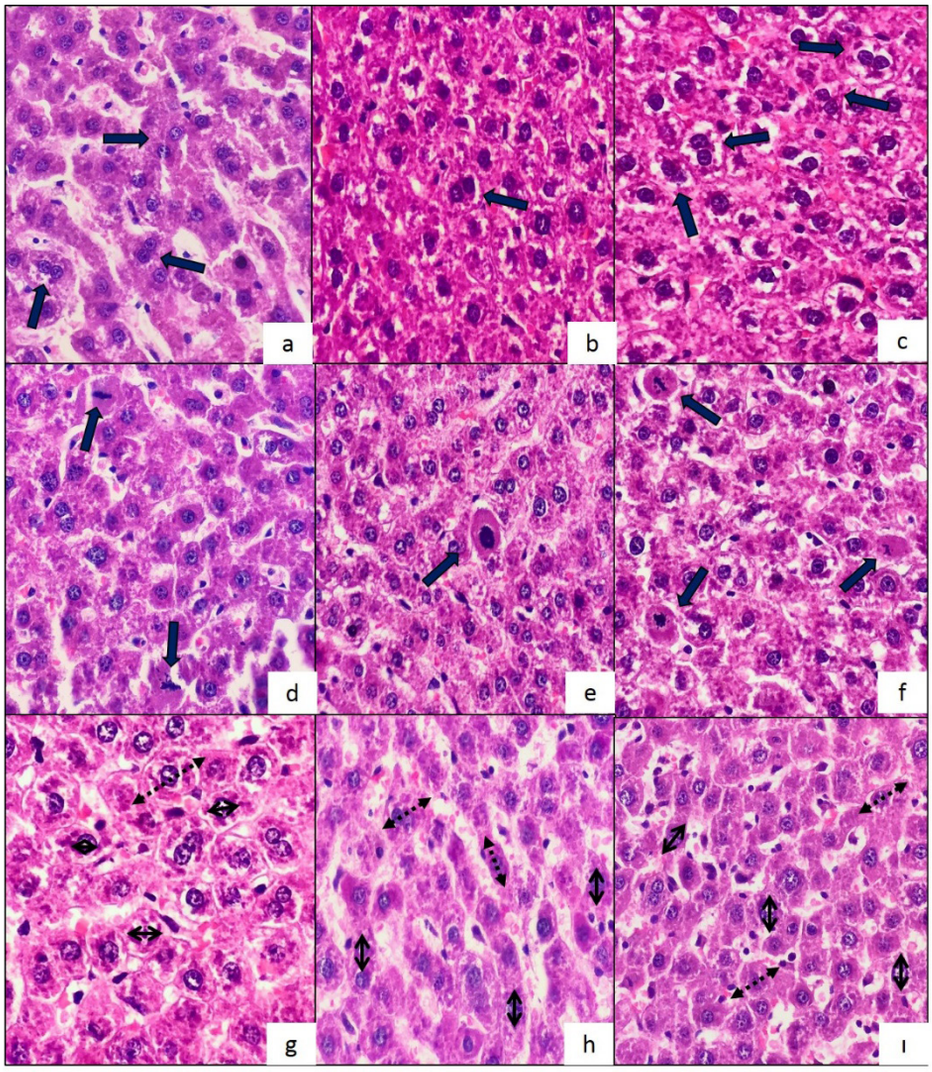

Figure 1. *Histomorphological findings of each group and also their comparison with each other (HEx400). Figures 1a-c show binucleation rates for group C, S and CS, respectively. Figures d-f show the mitotic figure for each groups in the same order. Group CS have the most mitotic figure among the groups as we see in figure f. Figures g-ı demonstrate the nuclear and hepatocyte size.*

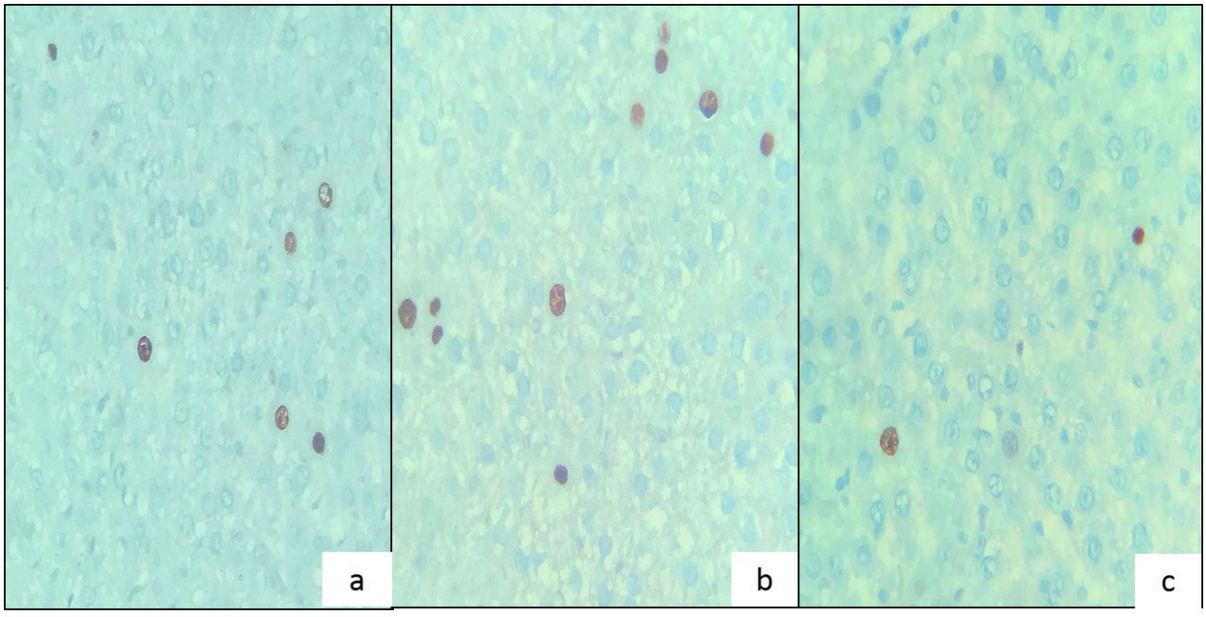

Figure 2. *a-c Ki67 proliferation index in groups C, S and CS, respectively (Ki67 immunohistochemistry x400).*

Table 2. *The comparison of histological changes in liver regeneration between groups (stained with H&E).*

Histological parameters (mean±SD)	C Group	S Group	CS Group	P
Size of nucleus (µm)	7.75 ±0.2	8.33±0.75	**10.4±0.6**	**<0.001**
Size of hepatocyte (µm)	315.9±80	265.8± 19	**480.6 ±38**	**<0.001**
Mitotic index (MI)%	2.25±1.3	1.5±0.7	**4.7±1.2**	**<0.001**
Binucleation rate (BR)%	6.5 ±2.1	12±5	**29.2±6**	**<0.001**
Ki 67 proliferation index (%)	2%	3 (%	**8 %**	**<0.001**

4. DISCUSSION

Currently, the main obstacle in liver transplantation is organ shortage. In order to overcome this obstacle, LDLT has been accepted as an option, particularly in Eastern countries, but the rate has slightly increased in Western countries as well. The main concern in LDLT is providing enough functional liver mass to maintain liver function in both the donors and recipients. The main success behind LDLT is liver regeneration. Liver regeneration not only makes LDLT available to those who need it, it also provides an option to surgeons to perform liver resection for advanced liver cancers that require extensive liver regeneration. However, it carries the risk of the remnant liver mass not being enough to maintain liver function. Both LDLT and liver resection for liver cancer rely upon liver regeneration to get enough liver mass (12).

Various strategies have been developed in models of hepatectomy to increase liver regeneration after hepatectomy in both humans and rats (13, 14). This study was performed to evaluate the role of CS in liver regeneration. It has been reported that CS has many health benefits (15), such as antioxidant activity. For example, one study reported that CS has liver-protective effects and downregulates oxidative stress in acute DZN-induced liver injury in rats (16). Another study showed liver-protective effects in liver damage induced by paracetamol (17). Nevertheless, research has still been needed to fully understand the effects of CS on liver regeneration after partial hepatectomy.

Although the normal liver is dormant with only a little hepatocyte proliferation, after partial hepatectomy, it was shown that the remaining liver undergoes a series of rapid endothelial, inflammatory, and epithelial changes (18). Multiple important mechanisms and factors control normal liver regeneration, such as IL-6, TNF, hepatocyte growth factor (HGF), epidermal growth factor (EGF), and thyroid hormone, which have been previously reported in detail and are not discussed here in depth (18, 19). The novel findings in this study are the CS-related histological changes of the liver after hepatectomy in rats. The changes were found to involve significant increases in the mean nucleus size, hepatocyte size, MI, BR, and Ki 67 proliferation index. Although changes were noted in all rats after partial hepatectomy compared to the control group, they were significantly higher in the CS group.

A previous study reported that by 7–10 days after hepatectomy, a rat's liver largely regrows to about normal size (93%) through hyperplasia of the remnant lobes (18). In this study, by 14 days, the average size of the liver at the end of the study in the sham group was 94.9% of the native liver size, but the recovery was higher (97.8%) in the CS group. The reason why there was not a significant difference between groups could be that the time needed to complete all regeneration is reported to be about 20 days (18). A second reason could be that we did not do 70% liver resection, as was done in a previous study; instead, we performed 22.2% resection (18).

It has been reported that the proliferation of hepatocytes induces regeneration of the liver (20). Proliferation and hypertrophy almost equally contribute to regeneration (21). The sizes of hepatocytes and nuclei are reported to increase significantly in regeneration (21, 22). This is consistent with our study, which showed increased hepatocyte size and nuclear size, and the changes were significantly higher in rats receiving CS. The number of binuclear hepatocytes and binuclear-to-total hepatocyte ratio are considered as signs of liver regeneration in other studies (22). However, the present study was not consistent with earlier studies, and we found that even the BR was higher in both the sham group and CS groups, but it was significantly higher in rats receiving CS.

A wide range of markers are used to describe hepatic regeneration criteria (18, 23). MI, which is used in the present study, is the ratio between the number of cells in mitosis and the total number of cells. Our study showed that MI was significantly higher in rats receiving CS. Previous studies reported using immunofluorescence staining of Ki-67 as a proliferation marker (21, 24). Ki-67 is expressed from the G1 to the M phase. Its expression was found to be higher in both the sham and CS groups compared to the control group. However, the expression of Ki-67 was significantly increased in rats that received CS compared with rats that did not.

CS is cultivated in many parts of the world because of its nutritional benefits and medicinal properties (25). It has shown beneficial effects in diseases of the biliary tract, digestive action, scurvy, anemia, and atherosclerosis (26, 27). However, this study is the first to our knowledge to demonstrate experimentally that CS increases liver regeneration after liver resection in rats.

There are some limitations in this study. We found out that CS increases liver regeneration by changing hepatocyte histology, but we did not investigate the exact mechanism of CS that creates the histological changes. Thus, studies are needed for further investigation. In conclusion, the present study showed that CS increases liver regeneration after partial liver resection with increases in both proliferation and hypertrophy in the remnant liver.

Acknowledgment

We would like to thank Veterinary Surgeon Osman Cengil for his great assistance in laboratory. This study was not financially supported by any institution or company

All authors declared no conflict of interest and funding.

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How to cite this article: Gunay Y, Kaymaz E. Cynara Scolymus (Artichoke) Improves Liver Regeneration after Partial Liver Resection in Rats. Clin Exp Health Sci 2022; 12: 653-658. DOI: 10.33808/clinexphealthsci.1008534