The effects of *Crataegus aronia* var. *dentata* Browicz extract on biochemical indices and apoptosis in partially hepatectomized liver in rats

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Abstract

Crataegus species have been widely used in herbal medicine, especially for the hearth diseases. In the present study, the effect of *Crataegus aronia* var. *dentata* Browicz extract on partially hepatectomized rats was investigated with biochemical and TUNEL apoptosis assays.

The extracts of the plant at the concentrations of 0.5 and 1 ml/100 g body weight/day were administered orally to the two experimental groups including partially hepatectomized rats for 42 days. At the end of the experimental period, animals were sacrificed, blood was collected for the assessment of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT), and the liver tissue was used for TUNEL assay.

In biochemical assay, it was found a significant decrease in the levels of serum ALT and AST in the experimental groups. On the other hand, the plant extract did not cause any significant changes in the level of GGT in these groups. In apoptosis assay, TUNEL positive hepatocytes could not be detected in both experimental groups.

The present findings can suggest that *Crataegus aronia* var. *dentata* Browicz extract can decrease the levels of serum ALT and AST and play a role in apoptosis of hepatocytes in the liver of partially hepatectomized rats. However, further studies are required to confirm the effects of the plant extract on hepatoprotection and apoptosis in the regenerating liver after partial hepatectomy in animal models.

KEY WORDS: *Crataegus aronia* var. *dentata* Browicz, liver, hepatectomy, apoptosis

INTRODUCTION

Liver can rapidly regenerate itself after acute liver injury, chronic hepatic diseases, liver transplantation and partial hepatectomy. Hepatectomy, characterized by increased apoptosis, induces oxidative injury in hepatocytes. Apoptosis (programmed cell death) is an important process during normal development and regeneration of the liver. Due to the significant roles in development, regeneration and tissue homeostasis, it plays a critical role in the balance between regeneration and cell death. Recently, many researches have received a great deal of attention about evaluating apoptotic cell death and the corresponding intracellular signaling pathways [1, 2, 3, 4, 5]. Besides, the importance of cell apoptosis in normal liver development, regeneration and also in hepatopathology is exclusively investigated [6, 7, 8, 9, 10, 11, 12, 13].

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Apoptosis is morphologically characterized by some cellular changes including DNA fragmentation and the appearance of cytoplasmic apoptotic bodies. As an important method for the demonstration of apoptotic cells, the TUNEL (deoxynucleotdyltransferase-mediated dUDP nick end labeling) method is based on the specific detection of DNA fragmentation in the nuclei of apoptotic cells [14]. In the past decade, there has been a great deal of interest in the use of medicinal plants for the treatment of diseases. Many researches have focused on the therapeutic properties of medicinal plants such as hepatoprotective, antioxidative, antimicrobial and gastric effects [7, 15, 16, 17, 18, 19, 20, 21]. Furthermore, many scientific studies have been extensively carried out by using medicinal plants in liver damages [15, 22, 23, 24, 25, 26]. *Crataegus* species, also known as Hawthorn, are medicinal plants, which have flavonoids, triterpene acids, proanthocyanidins, and organic acids as main components. Several studies demonstrated the cardioprotective effect of *Crataegus* spp [27, 28, 29, 30, 31]. Additionally, some studies have reported on different effectiveness of *Crataegus* spp such as antioxidant, antiinflammatory and anticarcinogenic effects [32, 33, 34, 35].

The aim of the present study was to investigate the hepatoprotective and apoptotic effects of *C. aronia* var. *dentata* Browicz extracts in the liver of partially hepatectomized rats using biochemical and TUNEL methods.

MATERIALS AND METHODS

Preparation of plant extract

C. aronia var. *dentata* Browicz grows along forest edges in Güzelpinar village, Denizli/Turkey. The flowers of the plants were collected and dried at shady places. Dried flowers were pulverized and put in the flask with 70% ethyl alcohol to let them dissolve until the concentration at 10%. The flask mouth was closed with a rubber stopper to protect the extract from contacting with air. The flask was covered with aluminum foil to protect the extract from light. Then, it was placed in the water bath at 55°C for six hours. The extract filtered and remaining sediment was dissolved with ethyl alcohol 70% and placed in the water bath at 55°C for another six hours. The extract was refiltered and the sum of the alcohol was evaporated using a rotary evaporator (Laborata 4010; Heidolph Instruments GmbH & Co. KG, Germany). Residual part of the extract was dissolved with ultrapure water and the water in the extract was lyophilized. Two different concentrations of the extract were prepared: 0.5% and 1%.

Treatment of animals

Male albino rats, weighing approximately 150-200 g, were obtained from the Pamukkale University, Faculty of Medicine, Experimental Research Center, Denizli, Turkey. The animals (Ethical approval was obtained from the Pamukkale University Animal Ethics Committee) were allocated into three groups with five rats in each group. Before the experimental period, 50% partial hepatectomy was performed under anaesthesia by removing the left lateral lobe from all the groups.

Group I: Control animals received normal rat diet and water, *ad libitum*.

Group II: The plant extract at concentration of 0.5 was given orally for 6 weeks.

Group III: The plant extract at concentration of 1 was given orally for 6 weeks.

After the experimental period, the animals were sacrificed under anesthesia, and blood samples were collected for the biochemical assays. For apoptosis assay, the livers were excised immediately from the animals, sliced thinly and embedded within optimal cutting temperature (OCT) compound. They were snap frozen in liquid nitrogen and stored at -86 °C for cryostat technique.

Biochemical assays

Blood samples were taken by cardiac venipuncture on the 2nd, 4th and 6th weeks after the initial treatment. Then, they were centrifuged at 1000 rpm for 10 minutes to collect serum and were stored at -20°C. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were measured for the determination of liver function.

Apoptosis assay

In order to detect the apoptotic cells, TUNEL assay on frozen tissue sections (6 µm) were performed using *In Situ* Cell Death Detection Kit (POD, ROCHE, cat: 11684817910). *Controls of TUNEL*: TUNEL controls were performed by incubating slides with 100 µl label solution.

Photography

Olympus BX50 light microscope and Olympus DP2-BSW microscope digital camera system were used for photography.

Statistical analysis

The biochemical results were evaluated using Kruskal-Wallis test in SPSS 15.0 program. The level of significance was set at $p<0.05$.

RESULTS

Biochemical findings

Tables 1, 2, and 3 give the mean serum AST, ALT and GGT levels for 2, 4, and 6 weeks in all groups. There was a significant difference in serum AST and ALT levels of the experimental groups when compared to controls ($p<0.05$). *C. aronia* var. *dentata* Browicz extract given to the hepatectomized rats significantly reduced the serum AST and ALT levels when compared to controls at the end of treatment. However, AST levels at the concentration of 0.5% were less than those of the levels of 1%. This effect appeared to be dose dependent. On the other hand, the extract did not affect the level of GGT.

TABLE 1. Serum AST, ALT and GGT levels of 2 weeks in partially hepatectomized rats

Groups		AST (IU/l)	ALT (IU/l)	GGT (IU/l)
Control		85.26±24.04	20.76±7.16	7.26±0.39
Experimentals	0.5%	34.52±9.64*	11.72±0.17*	7.28±0.38
	1%	59.82±24.21*	12.42±0.23*	7.48±0.31

*$p<0.05$ compared to control

TABLE 2. Serum AST, ALT and GGT levels of 4 weeks in partially hepatectomized rats

Groups		AST (IU/l)	ALT (IU/l)	GGT (IU/l)
Control		85.42±24.80*	21.56±7.77*	7.18±0.47
Experimentals	0.5%	32.60±8.79*	11.68±0.42*	7.10±0.52
	1%	59.48±24.66*	12.32±0.28*	7.44±0.49

*$p<0.05$ compared to control

TABLE 3. Serum AST, ALT and GGT levels of 6 weeks in partially hepatectomized rats

Groups		AST (IU/l)	ALT (IU/l)	GGT (IU/l)
Control		84.94±24.72*	20.08±6.92*	7.04±0.54
Experimentals	0.5%	34.36±9.56*	11.56±0.30*	7.22±0.13
	1%	59.70±24.29*	12.28±0.23*	7.48±0.17

*$p<0.05$ compared to control

Apoptosis assay findings

The plant extracts at concentrations of 0.5 ml and 1 ml were administered to the hepatectomized rats and the presence of apoptotic cells were investigated in liver tissue by TUNEL assay. Apoptotic cells were distinguished by distinct nuclear staining. In controls, positively stained nuclei were observed not only in hepatocytes, but also in sinusoidal cells (Figure 1b). However, TUNEL-positive hepatocytes could not be distinguished in the liver from 0.5% and 1% plant extract administered groups, whereas TUNEL-positive sinusoidal cells were observed (Figures 1c, d).

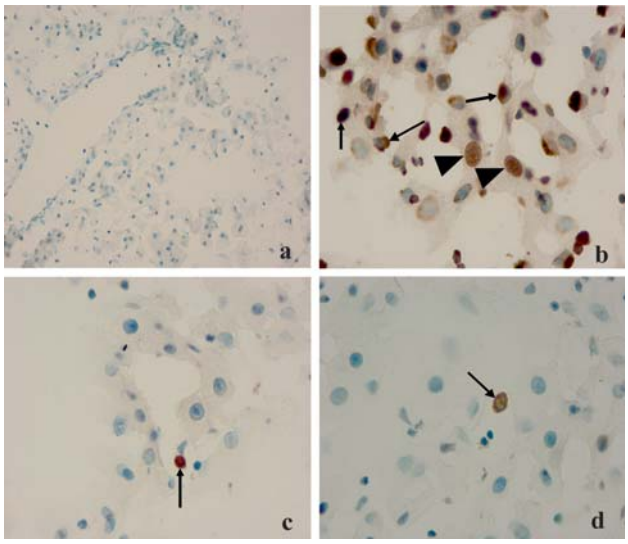

FIGURE 1. Tunel assay of hepatectomized liver in control (Group I) and experimental groups (Group II and III). a: negative control. b: TUNEL-positive hepatocytes (arrowheads), sinusoidal cells (arrows) were visualized in the Group I. c, d: It could not be distinguished apoptotic hepatocytes in the hepatectomized liver of experimental groups, but apoptotic sinusoidal cells were observed (arrows) (c: sections from the liver of Group II, d: sections from the liver of Group III), x1000

DISCUSSION

Crataegus species have received extensive attention in many scientific researches particularly on the treatment of cardiovascular diseases. In recent years, the effectiveness of the plant has been investigated on various disorders [36, 37]. Many researches have shown its antioxidant, cardioprotective, anticarcinogenic effects [27, 35, 38, 39, 40, 41, 42]. The major activity of the plant is thought to be provided by its flavonoid content. Flavonoids are biochemically active compounds with antiinflammatory and anticarcinogenic properties [43]. They have extensive biological properties that promote human health and help reduce the risk of disease [44]. As it is known, the values of ALT and AST are used as biochemical markers of liver damage [45]. From the biochemical analyses, *C. aronia* var. *dentata* Browicz extract in different concentrations administration lowered ALT and AST levels in partially hepatectomized rats. Particularly, there was a significant decrease of AST level at the concentration of 0.5% compared to that of 1%. This finding showed that the extract of *C. aronia* var. *dentata* Browicz may have protective effect against partial hepatectomy-induced liver injury. It has been concluded that fatal hepatic failure after excessive hepatectomy was characterized by increased apoptosis and diminished liver regeneration [46]. It has been reported that carvacrol obtained from *Origanum onites* L. (Lamiaceae) plant oil increases the liver regeneration rate following hepatectomy in rats [47]. In this study, the extracts of *C. aronia* var. *dentata* Browicz may increase the regeneration rate in hepatocytes in comparison to control group in the liver of partially hepatectomized rats. Furthermore, Swaminathan et al. [48] reported that *C. oxycantha* extract may reduce the oxidative stress in the reperfused myocardium, and play a significant role in the inhibition of apoptotic pathways leading to cardioprotection. Taken together, it is speculated that *C. aronia* var. *dentata* Browicz extract may play a role in the process of hepatocyte apoptosis and subsequent regeneration in hepatectomy-related injury.

CONCLUSION

In conclusion, *C. aronia* var. *dentata* Browicz extract may have potent antioxidant activity, reflected by reduced serum AST and ALT levels in partially hepatectomized rats. The mechanism of antioxidant activity may be explained by the flavanoids present in the extract. Moreover, the plant extract may enhance regenerative capacity of hepatocytes in experimental groups. Therefore, it can be suggested that *C. aronia* var. *dentata* Browicz extract may play a role in the apoptotic process of regenerating hepatocytes as well as in the antioxidant process after partial hepatectomy in rats. However, further studies are required to confirm the effects of the plant extract on hepatoprotection and the mechanisms of apoptosis and regeneration after partial hepatectomy in animal models.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

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